LEARNING GUIDE

CLINICAL CHEMISTRY

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INTENDED AUDIENCE

This Learning Guide is intended to serve the basic educational needs of new medical laboratory scientists who are entering the field of clinical laboratory medicine. Anyone associated with the specialty of clinical chemistry or the clinical laboratory will find this Learning Guide of interest. Typical readers will include medical laboratory technicians and medical technologists, laboratory supervisors and managers, nurses, laboratory support personnel and physician office laboratory personnel.

HOW TO USE THIS LEARNING GUIDE

To offer the most benefit, this Learning Guide begins each section with an overview so you can quickly review its goals and content. Next is a set of learning objectives. These focus on the key concepts presented in each section. There is a short review quiz at the end of each section designed to help recall the concepts introduced. If a question is answered incorrectly, the appropriate portions of the text may be reviewed before moving to the next section.

A glossary is at the end of this Learning Guide for quick reference. There are also references and resources devoted to other recommended reading for more details and further study.

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FOREWORD

Clinical chemistry is an exciting field that combines analytics and instrumentation with information technology and management of workflow, staff efficiencies and high volume automation. The field is ever-changing and demands staff have skills in the methodologies and their limitations, technology and troubleshooting equipment, as well as management and ability to adapt operations to evolving clinical needs. At the heart, the laboratory is a service to the physician providing test results that are critical to diagnosing and managing patients. But the laboratory is also a vital member of the healthcare team and gets involved in utilization, operational efficiencies and improving patient outcomes. I was honored to be asked to edit this version of the *Clinical Chemistry Learning Guide*, as I have spent nearly 30 years in the clinical laboratory mentoring students and helping physicians meet the needs of their patients.

This Learning Guide is an essential primer to the basics of not only clinical chemistry but laboratory medicine. It touches on quality and essentials of the methodologies we utilize in the daily analysis of specimens. A wide range of clinical disciplines, including nursing students, researchers, medical students, residents, lab administrators and government inspectors, as well as medical technologists, may find the contents of this guide to be helpful to their daily work and an explanation of what goes on in the clinical laboratory to those that are not aware of the science. I hope that you will find this guide as useful as my students and technologists have in the past.

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INTRODUCTION

CLINICAL CHEMISTRY LEARNING GUIDE

Clinical laboratory science consists of various specialties such as clinical chemistry, hematology, immunology, microbiology, serology, toxicology and urinalysis. This Learning Guide focuses on the major specialty of clinical chemistry, which encompasses a wide variety of tests and is a major area of concentration in hospital and reference core laboratories. Clinical chemistry uses many different methodologies and manual and fully automated tests; examines both very common and esoteric analytes; mixes basic chemistry with biochemistry, engineering, informatics and other disciplines; and overlaps with other areas of concentration, in particular, toxicology and endocrinology.

Because of the scope and depth of clinical chemistry, many excellent textbooks have been written on the subject. Those textbooks are routinely revised and updated to keep pace with developments in this dynamic field. This Learning Guide is only intended as a primer on the subject. It introduces basic concepts and is intended to provide the minimum fundamentals. It is hoped that this guide will answer elementary questions about clinical chemistry and stimulate further interest in the subject. Readers are encouraged to consult Appendix B: References for more comprehensive and detailed information about this specialty.

We hope this *Clinical Chemistry Learning Guide* from Abbott proves to be a useful tool to help you establish firm footing in the field of laboratory medicine.

SECTION 1

INTRODUCTION TO CLINICAL CHEMISTRY

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LEARNING OBJECTIVES

After completing this section, you will be able to:

1. Describe the kinds of analytes that are measured using clinical chemistry tests.
2. Identify different types of biologic specimens that may be used for testing.
3. Describe how the results of tests are interpreted.

OVERVIEW

This section identifies the scope of clinical chemistry testing, including the types of biologic samples that are typically analyzed and how test results may be interpreted.

KEY CONCEPTS

1. Clinical chemistry tests measure concentrations or activities of substances (ions, molecules, complexes) in body fluids.
2. These tests may use different kinds of body fluids such as whole blood, plasma, serum, urine and cerebrospinal fluid.
3. The medical interpretation of a test result is based on comparison to a reference interval that typically reflects the range of values expected for healthy people or a medical decision level (MDL) for the diagnosis and treatment of disease.

The reader should consult Appendix B: References for more detailed information about these topics, especially *Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics*, 7th edition, 2015, and the Lab Tests Online® website (www.labtestsonline.org).

Clinical chemistry is a quantitative science that is concerned with measurement of amounts of biologically important substances (called analytes) in body fluids. The methods to measure these substances are carefully designed to provide accurate assessments of their concentration. The results of clinical chemistry tests are compared to reference intervals or a medical decision level (MDL) to provide diagnostic and clinical meaning for the values.

COMMON ANALYTES

Clinical chemistry is the branch of laboratory medicine that focuses primarily on molecules. The tests in a clinical chemistry laboratory measure concentrations of biologically important ions (salts and minerals), small organic molecules and large macromolecules (primarily proteins). See Section 6 for more detail about specific analytes.

Common analytes in the clinical chemistry laboratory

Ions, salts and minerals	Small organic molecules	Large macromolecules
Potassium	**Metabolites**	**Transport proteins**
Sodium	Glucose	Albumin
Calcium	Cholesterol	Transferrin
Chloride	Urea	Haptoglobin
Magnesium	Lactic acid	Ferritin
Phosphorus	Bilirubin	Total protein
Carbon dioxide (CO_2)	Creatinine	**Enzymes**
Lead	Triglycerides	Lipase
Iron	Ammonia	Amylase
	Cystatin C	Alanine aminotransferase (ALT)
	Therapeutic drugs	Aspartate aminotransferase (AST)
	Vancomycin	Alkaline phosphatase (AlkP)
	Theophylline	Lactate dehydrogenase (LD)
	Digoxin	Creatine kinase (CK)
	Phenytoin	**Specific proteins**
	Valproic acid	Immunoglobulins (IgA, IgG, IgM)
	Toxicology	Complement C3
	Alcohol (ethanol)	Complement C4
	Salicylate (aspirin)	C-reactive protein (CRP)
	Acetaminophen	**Lipoproteins**
	Drugs of abuse (DOA)	High density lipoprotein (HDL)
	Cocaine	Low density lipoprotein (LDL)
	Barbiturates	Lipoprotein(a)
	Amphetamines	**Diabetes marker**
	Opiates	Hemoglobin A1c (HbA1c)
	Cannabinoids	

COMBINATIONS OF TESTS (PANELS)

When an individual test alone is not sufficient to assess a medical condition, a combination of several tests may be used. The pattern of results from the combination of tests may provide better insight into the status of the patient than any single test result. Such tests, done on the same sample, are often ordered as a group called a panel or profile.

The types of panels and the specific tests included in panels reflect local, regional or national practices. Even for panels with the same name, the individual tests included may differ from institution to institution.

Examples of typical panels of tests

Electrolyte panel	Hepatic panel (liver profile)	Comprehensive metabolic profile
Sodium (Na)	Albumin	Sodium (Na)
Potassium (K)	Total protein	Potassium (K)
Chloride (Cl)	Alkaline phosphatase	Chloride (Cl)
Carbon dioxide (CO_2)	Alanine aminotransferase (ALT)	Carbon dioxide (CO_2)
	Aspartate aminotransferase (AST)	Glucose
	Total bilirubin	Creatinine
	Direct bilirubin	Urea
		Calcium
		Total protein
		Albumin
		Alanine aminotransferase (ALT)
		Aspartate aminotransferase (AST)
		Alkaline phosphatase (AlkP)
		Total bilirubin

Basic metabolic panel	Lipid profile
Sodium (Na)	Total cholesterol
Potassium (K)	LDL cholesterol
Chloride (Cl)	HDL cholesterol
Carbon dioxide (CO_2)	Triglycerides
Glucose	
Creatinine	
Urea (blood urea nitrogen [BUN])	

BIOLOGIC SPECIMENS

Blood is the most common biologic fluid collected for clinical laboratory testing. It is usually drawn from a vein (in the arm) directly into an evacuated tube. Typically, a tube will hold about 5 mL of blood — enough to perform many clinical chemistry tests, since automated analyzers require only small amounts (usually from 2 to 100 μL) for a single test. Occasionally, when collection of blood from a vein is difficult, a sample of capillary blood may be collected by pricking the skin and collecting several drops of blood from the puncture site. An example is the use of heelstick blood for testing of newborns.

> **Phlebotomy** is the act of drawing a blood sample from a blood vessel. For clinical chemistry testing, blood is usually drawn from a vein, typically a vein in the arm or back of the hand. Collecting blood from a vein is called **venipuncture**. The medical professional drawing the blood sample is called a **phlebotomist**.

Other biologic fluids (matrices) often used for testing include urine, saliva, cerebrospinal fluid (CSF), amniotic fluid, synovial fluid, pleural fluid, peritoneal fluid and pericardial fluid. These fluids often contain the same biologic analytes of interest — such as glucose and protein — but differ greatly from each other in physical and chemical properties. These differences in fluid characteristics are termed matrix differences. Test methods that are designed for determination of an analyte in blood plasma may not be suitable for determination of that same analyte in other fluids (other matrices). When using a test method for analysis of a fluid other than blood plasma or serum, it is important to validate that the method is acceptable for the type of fluid sample being used.

Fluids typically used for clinical chemistry tests
Blood (whole blood, serum or plasma)
Urine
Cerebrospinal fluid (CSF)
Amniotic fluid
Saliva
Synovial fluid (fluid that is found in joint cavities)
Pleural fluid (from the sac surrounding the lungs)
Pericardial fluid (from the sac surrounding the heart)
Peritoneal fluid (also called ascitic fluid; from the abdomen)

BLOOD

Blood is the most commonly used specimen for testing in the clinical laboratory. Blood consists of two main parts — a fluid portion (called plasma, which contains the dissolved ions and molecules) and a cellular portion (the red blood cells, white blood cells and platelets). Most clinical chemistry analytes are found in the plasma. Part of the preparation of blood for testing these analytes involves removing the cells. This is done by centrifugation of the sample to pack the blood cells in the bottom of the collection tube and allow removal of the liquid portion for testing.

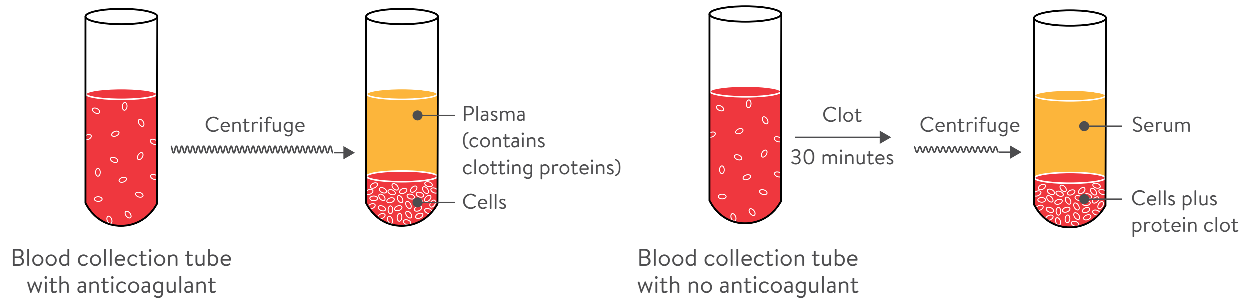

Figure 1-1: Preparation of serum and plasma.

If a blood sample is collected in a tube containing an additive that prevents the blood from clotting (called an anticoagulant), the fluid portion of the blood is called **plasma**. If the blood is collected in a tube with no anticoagulant, the blood will form a clot. A clot is a gelatinous semisolid composed of cross-linked protein that is formed in a multistep process referred to as the clotting cascade. Upon centrifugation, the clot descends to the bottom of the tube along with the cells. The resultant liquid above the cells and clot is called **serum**. Serum contains all the components of plasma except the clotting proteins, which are consumed in the cascade of reactions that form the blood clot.

Some clinical chemistry tests are best performed using plasma, others are best performed using serum, and still others can be performed using either plasma or serum.

Tubes used to collect blood have color-coded caps that signal what, if any, additives are present in the tube. Additives may be anticoagulants to allow preparation of plasma or may be substances included to protect analytes from chemical or metabolic breakdown.

Note: Certain types of anticoagulants may be incompatible with some kinds of tests. For example, EDTA is an anticoagulant that inhibits the clotting of blood by sequestering calcium ions that are necessary components of clotting reactions. However, samples of plasma collected using EDTA tubes are generally unsuitable for measurement of calcium and for any test method that involves a reaction step depending on availability of calcium.

Types of blood collection tubes commonly used for chemistry tests*

Tube additive**	Stopper color	Sample	Comment
None	Red	Serum	Clotting requires at least 30 minutes at room temperature
Silica clot activator	Red/black	Serum	Silica speeds the clotting process compared to no activator
Thrombin	Gray/yellow	Serum	Speeds clotting process significantly to produce serum in several minutes — used mostly for urgent (STAT) testing
Lithium heparin	Green	Plasma	Preferred plasma sample for most chemistry tests — not suitable when testing for lithium
Sodium heparin	Green	Plasma	Used when testing for lithium — not suitable when testing for sodium
EDTA (ethylenediaminetetraacetic acid as sodium or potassium salt)	Lavender	Plasma	Occasionally used for some chemistry tests and typically used for hematology
Potassium EDTA in special plastic tube	Tan or brown	Plasma	For blood lead testing; tubes are certified to have very low levels of contamination by lead
Sodium fluoride/potassium oxalate	Gray	Plasma	For glucose testing — sodium fluoride inhibits metabolism of glucose by white blood cells

*For more information, see www.bd.com/vacutainer.

**Some collection tubes also contain an inert silica gel that positions itself between the cells and the serum or plasma during the centrifugation step. It seals the cells in the bottom of the tube and prevents substances that leak from the cells from contaminating the serum or plasma. These are called serum separator tubes (designated SST) or plasma separator tubes (designated PST).

URINE

Urine is another fluid commonly used for testing in clinical chemistry laboratories. It is especially suitable for tests that evaluate kidney functions, for tests that look at waste products that are excreted by the kidneys, and for metabolites that are cleared quickly from the bloodstream and accumulate in the urine, such as drugs of abuse. Sometimes both serum and urine concentrations of a substance are useful to know to evaluate how well the analyte is being excreted — either to ensure that expected excretion is taking place or to determine if unexpected leakage is occurring.

Urine samples can be concentrated or dilute depending on the hydration status and kidney function of the patient. These differences in urine can affect the amount of a substance found in a sample at different times. Since creatinine is excreted at fairly constant rates over time, urine analytes are sometimes normalized to the amount of creatinine in the sample to correct for the differences in the hydration state of the patient and concentrated versus dilute samples.

Urine is relatively easy to collect from most people, although special techniques may be needed for infants and small children. Different types of urine samples, representing collection at different times of day and for different durations of time, are used for laboratory analyses.

Type of urine sample	How it is used
First morning sample	Provides a concentrated sample of urine that contains the overnight accumulation of metabolites. Useful for detection of proteins or unusual analytes.
Random	Convenient sample that can be collected at any time. Most often used for routine screening tests.
Timed	Typically, 2 to 6 hours of urine output are collected to give a representative sample; duration of collection depends on the analytes.
24-hour	Entire urine output for a 24-hour period is collected. Like a timed urine, but used for metabolites whose excretion rates may vary with time of day and full 24-hour collection is needed to be representative.

Often, when urine samples will not be tested immediately upon collection, the urine must be treated with a preservative. A preservative is a substance that prevents the breakdown of analytes of interest. Most preservatives are added to reduce bacterial metabolism or to prevent chemical decomposition of the analyte(s) of interest. This is typically done by adjusting the pH to an acidic or basic range. Some of the common urine preservatives include potassium phosphate, benzoic acid, sodium bicarbonate, acetic acid, hydrochloric acid and boric acid.

OTHER FLUIDS

Fluids other than blood and urine, like amniotic fluid, synovial fluid, peritoneal fluid, pleural fluid and pericardial fluid, are used in limited clinical settings and are tested for only a few special analytes.

Amniotic fluid is typically used for tests of fetal health. Spinal fluid is used primarily for assessment of patients with symptoms of diseases such as meningitis or multiple sclerosis or patients who may have suffered a cerebrovascular accident. Chemical testing of fluids such as peritoneal fluid, pericardial fluid or pleural fluid is typically done to assess the origin of the fluid — to determine whether it has leaked from blood vessels because of pressure differences (called a transudate, which is relatively low in protein) or because of inflammation or injury (called an exudate, which is relatively high in protein). Saliva is rarely used in clinical laboratory testing, but is recognized as a specimen whose composition reflects the blood plasma levels of many low molecular weight substances such as drugs or alcohol.

Saliva can be collected without the privacy concerns of observed urine collection for drugs of abuse testing — to witness the specimen collection and prevent sample adulteration or substitution by the patient. Saliva also has an advantage for hormones like cortisol for pediatric patients, when blood collection is too painful or stressful.

REFERENCE INTERVALS

Test results are generally expressed in numbers with units — reflecting the amount of analyte in a given volume of fluid (concentration). The results of a patient's test are compared to a reference interval, a range that has been documented to reflect the results expected for healthy people. There are several ways to define a reference interval. Some reference intervals are based on consensus values that reflect medical decision levels; these values are agreed upon by healthcare professionals as good indicators for medical decision-making. Some reference intervals, especially for tests where there is no medical consensus value, are based on statistical analysis of results of the test for healthy populations.

CONSENSUS REFERENCE INTERVALS

When test results can be correlated with medical decision levels (MDLs), reference intervals are determined by consensus of healthcare professionals. The values are based on the results of clinical research and clinical experience. For example, the American Diabetes Association® (ADA) has used results from many clinical research trials to develop consensus values for blood glucose and hemoglobin A1c. The American Heart Association® (AHA) and the Expert Panel from the National Cholesterol Education Program (NCEP) have evaluated the role of lipids as risk factors for heart disease and, based on many research studies of heart disease, have identified desirable ranges for cholesterol, triglycerides, HDL and LDL.

Examples of consensus reference intervals/medical decision levels for clinical chemistry tests

Analyte	Reference interval	Consensus group
Glucose (fasting)	< 100 mg/dL (< 5.5 mmol/L) nondiabetic 100–125 mg/dL (5.5–6.9 mmol/L) prediabetes ≥ 126 mg/dL (> 7.0 mmol/L) diabetes	American Diabetes Association® (ADA)
Cholesterol	Desirable — < 200 mg/dL (< 5.2 mmol/L) Moderate risk — 200–239 mg/dL (5.2–6.2 mmol/L) High risk — > 240 mg/dL (> 6.2 mmol/L)	American Heart Association® (AHA) and National Cholesterol Education Program (NCEP)
Triglycerides	< 150 mg/dL (< 1.7 mmol/L)	American Heart Association® (AHA) and National Cholesterol Education Program (NCEP)
Prostate-specific antigen (PSA)	< 4 ng/mL (< 4 µg/L)	American Cancer Society® (ACS)
Hemoglobin A1c	Normal < 5.7% (< 39 mmol/mol) Prediabetes 5.7%–6.4% (39–46 mmol/mol) Diabetes ≥ 6.5% (≥ 48 mmol/mol)	American Diabetes Association (ADA) (DCCT/NGSP)* and International Federation of Clinical Chemistry (IFCC)*

*American Diabetes Association bases its reference intervals on the results of the Diabetes Control and Complications Trial (DCCT) and the values standardized to the National Glycohemoglobin Standardization Program (NGSP). The International Federation of Clinical Chemistry (IFCC) recommends using reference intervals in mmol of hemoglobin A1c per mole of hemoglobin based on its standardization program.

STATISTICAL REFERENCE INTERVALS

When a test does not have a clear association with a single disease or condition, or when there is insufficient medical evidence to define a specific reference interval, a statistical approach is taken. The reference interval is typically based on the statistical distribution of values obtained when the test is done on hundreds of healthy people. The figure below is an example of the range of results that might be obtained for calcium.

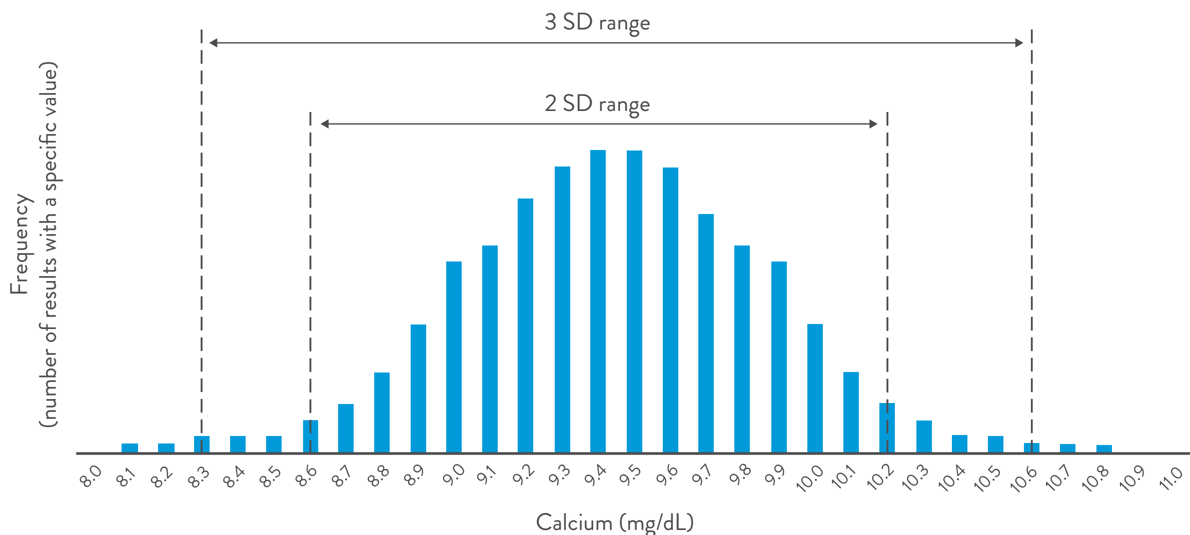

Figure 1-2: Calcium distribution in healthy adults.

The range of results identifies the values that are most often seen in healthy populations. For a typical bell-shaped (Gaussian) distribution, as shown for calcium, approximately 66% of all results are within one standard deviation (SD) of the mean (1 SD). Approximately 95% of all values are within two standard deviations of the mean, and approximately 99% of all values are within three standard deviations of the mean. The reference interval is generally chosen to capture the central 95% of healthy people and is set to be the range from –2 SD to +2 SD (referred to as a 2 SD range). Sometimes it is set to capture the central 99% of healthy people and is set to be the range from –3 SD to +3 SD (referred to as a 3 SD range). For the example given for calcium, a 2 SD reference interval would be 8.6–10.2 mg/dL, whereas a 3 SD reference interval would be from 8.3–10.6 mg/dL. In the first case, 5% or five out of every hundred healthy people would be expected to have a test result outside of the reference interval — either high or low. In the second case, only 1% or one out of every hundred healthy people would be expected to have a result outside the reference interval. Most chemistry tests use 2 SD reference intervals, while some tests, like troponin, use 3 SD reference intervals (99th percentile) to separate healthy patients from those with a disease.

Many analytes, however, do not demonstrate a normal Gaussian distribution in healthy populations. Values may be skewed toward one side of the mean or have extended high or low tails of values that distort the bell-shaped curve. In these instances, the reference interval can be estimated using nonparametric statistics that make no assumptions regarding the shape of the curve. In nonparametric analysis, result values are ranked from low to high, and 2.5% of values are excluded from each end to define the central 95% of values. A 2 SD reference interval is the values that encompass the central 95%, or mean +/- 2 SD, of the distribution of results of a healthy population regardless of whether the results are Gaussian (bell-shaped) or non-Gaussian (irregularly) distributed. Similarly, a 3 SD reference interval would encompass 99% of the distribution of results from a healthy population.

If the range of values seen in healthy people tends to be near zero, and there is no medical concern about low values, the reference interval is sometimes expressed as zero to a number that represents the upper limit of the 95th or 99th percentile of the healthy population. It may also simply be expressed as less than (<) that number.

Expected values may vary among different healthy populations, and different reference intervals are often reported for these populations. Most common differences are those based on gender, age or ethnicity. Specific population ranges are statistical ranges determined for each population based on the chosen partitioning factors.

Examples of a few reference intervals that vary for different populations

Analyte	Population	Reference interval*
Alkaline phosphatase	Age 20–50 years male	53–128 U/L (0.90–2.18 µkat/L)
	Age 20–50 years female	42–98 U/L (0.71–167 µkat/L)
	Age ≥ 60 years male	56–119 U/L (0.95–2.02 µkat/L)
	Age ≥ 60 years female	53–141 U/L (0.90–2.40 µkat/L)
Creatine kinase	Male	25–130 U/L (0.43–2.21 µkat/L)
	Female	10–115 U/L (0.17–1.96 µkat/L)
Urine creatinine	Infant	8–20 mg/kg/day (71–177 µmol/kg/day)
	Child**	8–22 mg/kg/day (71–194 µmol/kg/day)
	Adolescent**	8–30 mg/kg/day (71–265 µmol/kg/day
	Adult male	14–26 mg/kg/day (124–230 µmol/kg/day)
	Adult female	11–20 mg/kg/day (97–177 µmol/kg/day)

Note: Reference intervals may be found in clinical chemistry textbooks, on medical websites and in materials provided by manufacturers of tests and equipment. These will often have different values that reflect the methods and populations used in each setting. Since different laboratories use different methods and serve different populations, it is important for each laboratory to confirm that the reference intervals reported for their tests are appropriate. This is typically done by analyzing samples obtained from healthy people and demonstrating that the results coincide with the reported reference interval.

*Reference ranges or expected results for healthy adult individuals are provided as a guide for discussion in this section. These values were sourced from the 7th edition of *Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics*, unless otherwise stated. These values may differ with different patient populations, locale and assay methodologies and should be verified by laboratories prior to use.

**Determination and validation of pediatric reference ranges present a special challenge since blood is rarely drawn from healthy children. Literature citations that give both pediatric and adult ranges for a single test method may be helpful in evaluating tests that have separate pediatric reference intervals.

GUIDANCE ON ESTABLISHING REFERENCE INTERVALS

The Clinical and Laboratory Standards Institute® (CLSI®) publishes guideline C28, providing guidance for the establishment of reference intervals for clinical laboratory tests. More information may be found on the CLSI website at www.clsi.org.

LIMITATIONS OF REFERENCE INTERVALS

Reference intervals are best viewed as guidelines. When the value obtained for a test falls outside the expected reference interval, the unusual result serves as a signal that there may be a problem. The unusual result needs to be interpreted in the context of the patient's medical history and other clinical findings.

REVIEW QUESTIONS: SECTION 1

Answers are provided at the end of this Learning Guide.

1. Which of the following would be a typical analyte in a clinical chemistry test?

   A Calcium

   B *E. coli* positivity

   C Octane

   D Food additives

2. Name five kinds of body fluids that might be used for testing in a clinical chemistry laboratory.

   1 ______________________________

   2 ______________________________

   3 ______________________________

   4 ______________________________

   5 ______________________________

3. How should a laboratory verify the reference range it uses for a particular test?

   A Call another laboratory

   B Use the numbers from a textbook

   C Test samples from healthy people

   D Look on a medical internet site

4. Typically, a patient test result that exceeds 3 SD of the mean value for analyte is found with a frequency of:

   A 1 in 5

   B 1 in 20

   C 1 in 100

   D Never

5. What type of additive is in a blood collection tube with a red cap?

   A Lithium or sodium heparin

   B Potassium EDTA

   C Thrombin

   D No additive

SECTION 2

PRINCIPLES OF MEASUREMENT

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LEARNING OBJECTIVES

After completing this section, you will be able to:

1. Describe the basis for optical methods such as absorbance, turbidimetry and nephelometry.
2. Describe the difference between an endpoint and a rate reaction.
3. Describe the principle of potentiometric measurement.
4. Describe the role of calibrators.

OVERVIEW

This section describes measurement principles — optical (photometric) and electrochemical (potentiometric) — that are most often used to determine concentrations of analytes in the clinical chemistry laboratory.

KEY CONCEPTS

1. Chemical reactions of analytes produce products that can be detected by using optical methods; changes in light absorbed, scattered or emitted by these products are used to determine the concentration of the analyte.
2. In potentiometric methods, changes in concentrations of ions are sensed as potential differences between two electrodes.
3. Calibrators, solutions of known concentration, are used to establish the relationship between the magnitude of an optical or electrical signal and the corresponding concentration of analyte.

Quantitation of routine chemistry analytes is typically based on one of two measurement principles — measurement of light (photometry or spectrophotometry) or measurement of electrochemical potential (potentiometry). There are many variations of photometry and potentiometry, but all have in common that the signal — the amount of light or electrical voltage — is predictably related to the amount of analyte in solution.

The reader should consult Appendix B: References for more detailed information about these topics, especially *Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics*, 7th edition, 2015.

PHOTOMETRY

Photometry relies on measurement of light by a photodetector. The light may be absorbed by a substance dissolved in solution (absorbance), the light may be scattered or refracted by particles suspended in solution (turbidimetry or nephelometry), or the light may be emitted from a substance that absorbs light at one wavelength and emits light at another wavelength (fluorescence).

Specific wavelengths of light are chosen for each analysis based on the properties of the substance being measured. A typical light source (lamp) generates a broad range of wavelengths of light. A visible lamp produces light of wavelengths from 400 nm (violet light) to 700 nm (red light). An ultraviolet lamp produces light of wavelengths from about 200 to 400 nm. To select the desired wavelength from the spectrum of light produced by the light source, a device called a monochromator or filters are used. A monochromator disperses the light (much like a prism disperses light) and allows selection of a narrow band of wavelengths to be directed through the sample cuvette.

> A **cuvette** is a cell made of optically transparent material that contains solutions for analysis by optical methods

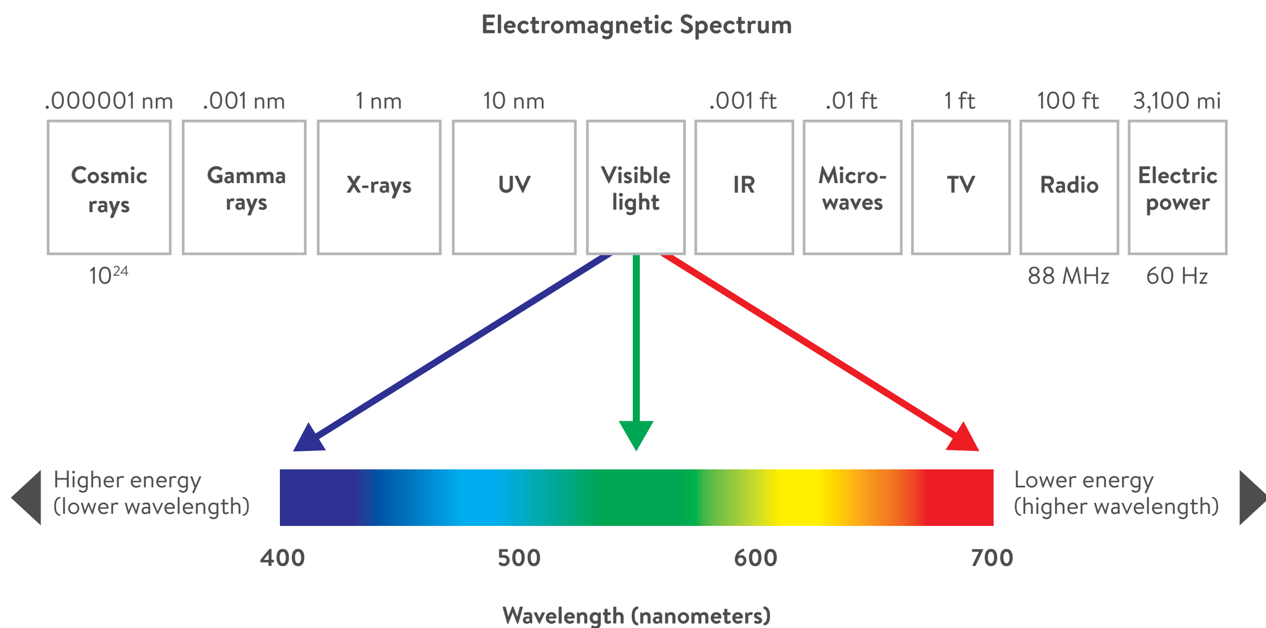


Figure 2-1: Electromagnetic spectrum.

ABSORBANCE

When an analyte has an intrinsic color (or generates a color upon chemical reaction), visible light is absorbed when it passes through a solution containing the analyte (or reaction products). The selective absorbance of certain wavelengths of light from the spectrum of white light gives the solution its color. For example, a solution containing hemoglobin appears red because light in the green range of the spectrum (wavelengths of 500–600 nm) is selectively absorbed (removed from the white spectrum). Measuring the decrease in green light that occurs upon passing through the solution gives an indication of the amount of hemoglobin present. **Figure 2-2** shows the configuration used to measure light absorbed — the difference between the emitted light from the source (I_o) and the light that reaches the photodetector (I_s).

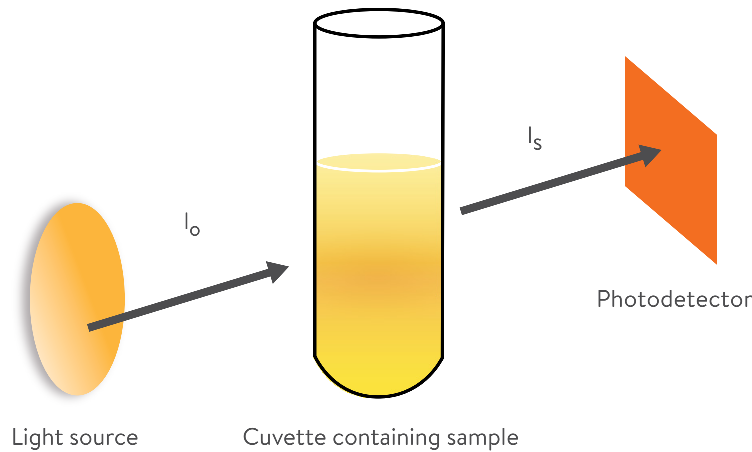


Figure 2-2: Absorption photometry.

Compounds that have no visible color often absorb light in the ultraviolet region, and this absorbance can be used in the same way as absorbance of visible light. The specific wavelength of light chosen is based on the absorption properties of the compound being measured.

As the amount of a substance in solution increases, the relative amount of light that passes through the solution and reaches the detector decreases. The decrease in light is termed absorbance. A formula called Beer's Law describes the relationship between concentration and absorbed light. For a given method, A = elc, where e is the extinction coefficient, l is the length of cuvette and c is the concentration.

Beer's Law
A = elc; e = extinction coefficient; l = length of cuvette; c = concentration

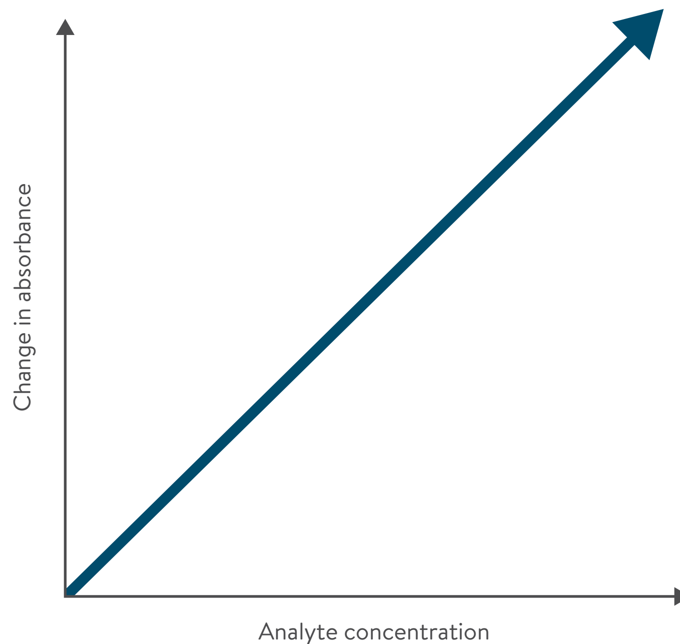


Figure 2-3: Beer's Law.

TURBIDIMETRY AND NEPHELOMETRY

Some tests are based on formation of insoluble particles that interfere with the passage of light through the solution. The analyte reacts with an added reagent to produce insoluble particles that remain suspended in the solution. When light hits these particles, some of it is reflected in different directions. A higher concentration of analyte presents a greater number of particles that will inhibit light passing through the solution and increase the amount of light reflected.

It is possible to measure the loss of light passing straight through the solution (called turbidimetry) or the increase of light reflected in a different direction (called nephelometry). In turbidimetry, the detector is placed in a direct line with the incident light and the light sensed by the detector decreases as the number of analyte particles increases. In nephelometry, the detector is placed at an angle to the light path to avoid detection of light passing through the sample. The nephelometric detector senses light scattered by the particles; the amount of light reaching the detector increases as the number of analyte particles increases. Often, antibodies are used with these methods and represent a type of immunometric assay, specifically, immunoturbidimetry and immunonephelometry. The antibodies in the reagents will cause analyte molecules to form complexes or lattices, and these large particle aggregates enhance the reflection of light, increasing the analytical signal that is measured.

Turbidimetric and nephelometric methods are often chosen to measure proteins such as transferrin or prealbumin — two important transport proteins in the blood. Proteins are relatively large molecules that can be easily cross-linked by selective antibodies to produce aggregate particles that are the right size to reflect light in the visible or ultraviolet range. Some drugs of abuse tests can quantitate the concentration of drug in the patient's sample by measuring the changes in turbidity due to the competition between drug in the patient's sample and drug bound to microparticles with antibodies added to the solution.

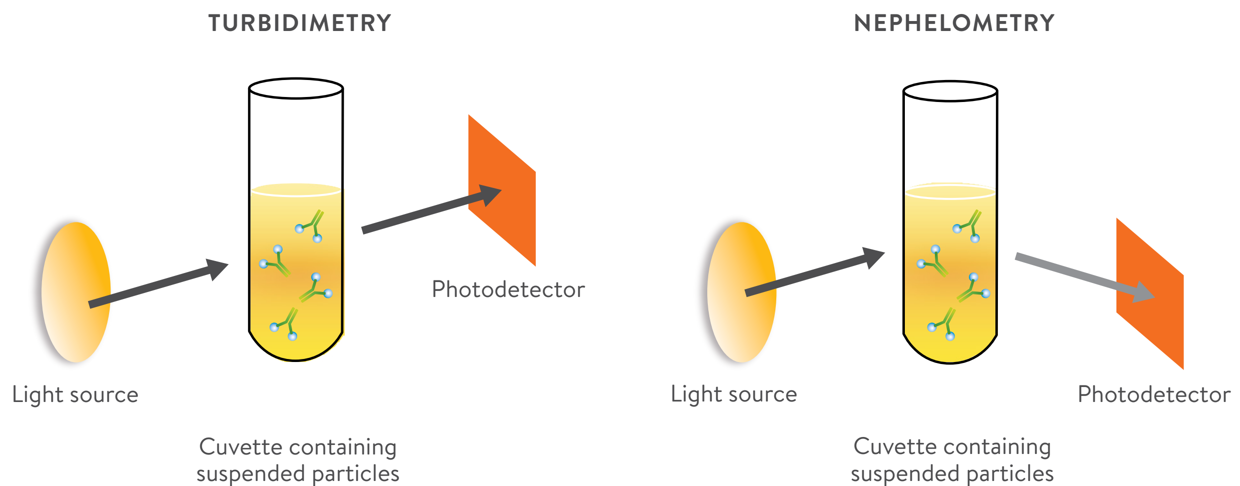


Figure 2-4: Turbidimetry and nephelometry.

FLUORESCENCE

Certain kinds of chemical structures are able to absorb light of one wavelength and emit light of another wavelength. These substances are termed fluorescent compounds or fluorophores. In each case, the incident light is of shorter wavelength and higher energy than the emitted light. So a substance that absorbs blue light (wavelength 400) may emit lower energy green light (wavelength 500).

The detector is placed at a 90° angle from the incident light so that it detects only emitted light and not residual incident light that passes directly through the sample, or reflected light bounded back from the sample or cuvette. The more light emitted by the sample, the greater the concentration of the fluorescent compound.

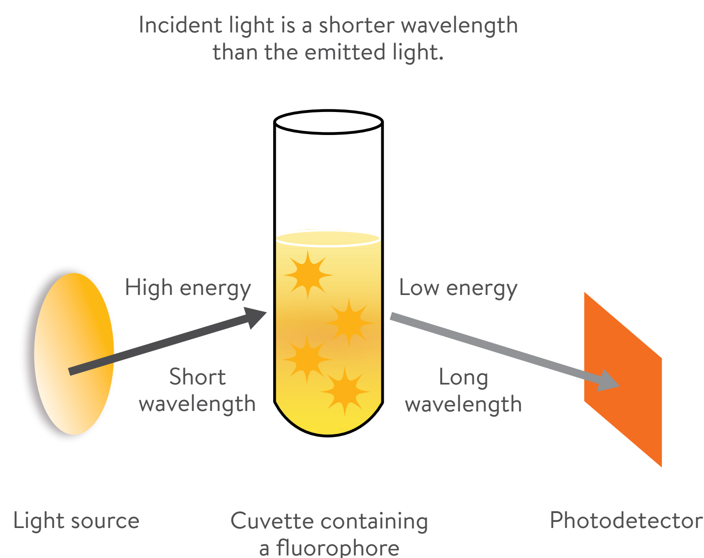


Figure 2-5: Fluorescent photometry.

Analytes of interest in clinical chemistry are not innately fluorescent. Instead, fluorescent molecules are incorporated as reagents to help detect analytes. For example, immunological methods for measurement of tumor markers like CA-125 (ovarian cancer marker) and CA15-3 (breast cancer marker) use antibodies that have a fluorescent compound attached. The antibody recognizes and binds to the tumor marker. Excess (unbound) antibodies are washed away, and the amount of fluorescent light generated is in direct proportion to the amount of tumor marker in the sample.

Chemiluminescence is light emission due to a chemical reaction. Similar to fluorescence, there are some molecules that, due to their structure, can produce light rather than heat when they react with other molecules. Some immunological methods for measuring hormones and tumor markers utilize a chemiluminescent molecule. After a selective antibody binds to the analyte in the patient's specimen, the bound and unbound antibodies are separated, and a second antibody linked to a chemiluminescent molecule is added. Once bound to the first antibody bound complex, a chemical is added to the mixture to generate a chemiluminescent signal that is proportional to the amount of analyte in the sample. In a variation of this technique, the chemiluminescent signal is generated by pulsing the mixture with an electrical current rather than through a chemical reaction. This method is called electrochemiluminescence.

POTENTIOMETRY

Potentiometry is based on measurement of an electrical potential between two electrodes. One of the electrodes (the measuring or sensing electrode) is affected by contact with ions in solution. The potential between the measuring electrode and a stable reference electrode is altered as the concentration of ions changes. Potentiometric methods are best suited for measurement of ions (electrolytes) such as sodium, potassium and chloride.

The voltage change is a complex function of the concentration of each ion and is described in a logarithmic relationship called the Nernst equation.

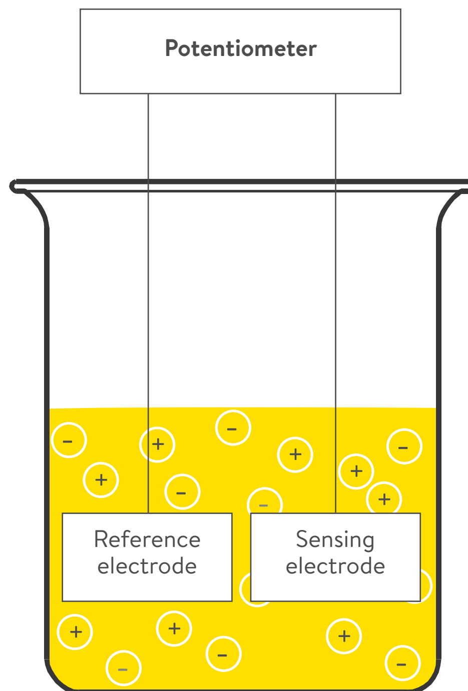


Figure 2-6: Electrolyte analysis.

Potentiometry measures the activity of the ion or effective concentration of the ion in the solution. Because ions interact with each other and with water in a real-world sample, clinical specimens are not ideal. Measuring the actual concentration of an ion requires an infinitely dilute solution where all of the ion interactions disappear. The difference between effective concentration that is measured by electrodes in the laboratory and the actual concentration in an ideal dilute solution is described by the activity coefficient. The activity coefficient is constant under specified conditions. However, differences in heat, pH, ionic strength and sample mixtures can alter the relationship between the measured activity and resulted concentration. So, many chemistry analyzers dilute the specimen into a solution of fixed ionic strength and perform the analysis under controlled conditions to minimize these sources of bias. This is termed an indirect method because the sample is diluted before the potentiometric measurement. Direct methods, such as blood gas analyzers, measure ion activity in whole blood and control conditions with a heat block.

ENDPOINT AND RATE REACTIONS

When an analyte is detected using a chemical reaction, there are two options for assessing its concentration. One is to wait until the reaction is complete and the total amount of analyte is converted to product (called an endpoint reaction). The other is to measure the rate of change in product formed over time (called a rate reaction).

ENDPOINT REACTIONS

Endpoint reactions are especially suitable for chemical reactions that are completed in a relatively short time and are "stoichiometric," meaning that they produce one product molecule or complex for each molecule of analyte. For example, a reaction of albumin with the dye bromocresol purple (BCP) produces a colored complex. If the reaction is allowed to continue until all the albumin present in solution has reacted and the maximum amount of colored product has formed, the color at the end of the reaction reflects the total amount of albumin as the albumin-dye complex.

Endpoint reactions can measure the creation of a product or the loss of reactant. If the method measures the creation of a product, the absorbance is higher at the endpoint than at the start point (called an end-up reaction). If the method measures the disappearance of a reactant, the absorbance is lower at the endpoint than at the start point (called an end-down reaction).

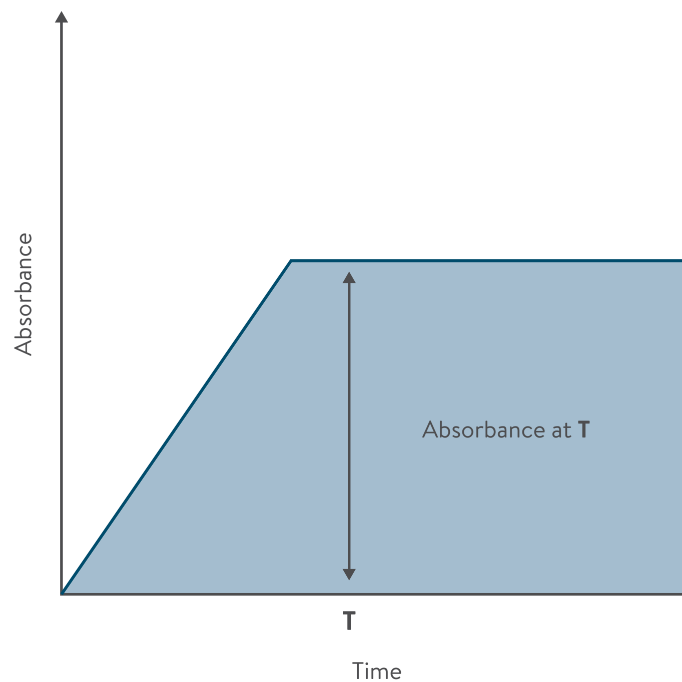


Figure 2-7: Endpoint reaction.

RATE REACTIONS

If the analyte is an enzyme, a molecule that can catalyze the conversion of unlimited numbers of reagent molecules (termed substrates) to product, the amount of product at the endpoint would not reflect the amount of enzyme. Instead, the endpoint would reflect the amount of substrate that was present. For this reason, enzyme activity is determined by a rate reaction rather than an endpoint reaction. In such cases, determination of the enzyme concentration is based on how fast a fixed amount of substrate is converted to product. The more enzyme present, the faster the conversion. Examples of enzymes that are often measured in the clinical laboratory include lipase (a digestive enzyme measured in pancreatic diseases) and alanine aminotransferase (an enzyme responsible for interconversion of amino acids measured in liver diseases).

Rate reactions can measure the appearance of a product or the disappearance of a substrate. If measuring the appearance of a product, the absorbance increases with time (called a rate-up reaction). If measuring the disappearance of a substrate, the absorbance decreases with time (called a rate-down reaction).

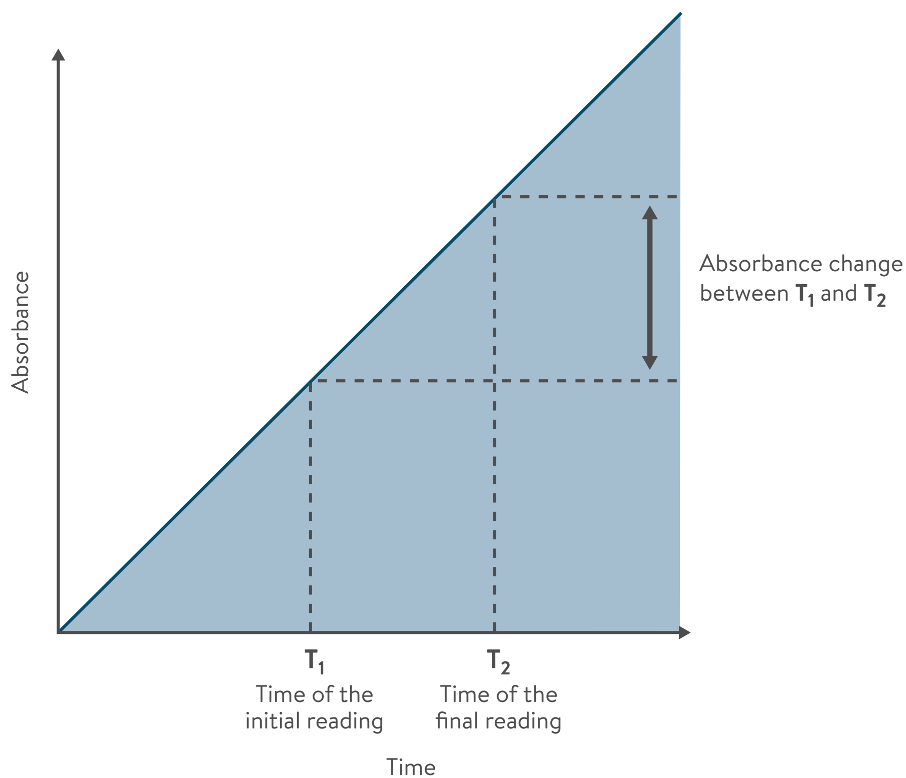


Figure 2-8: Rate reaction.

Rate reactions may also be used for measurement of analytes that are not enzymes. For example, if a reaction is very slow to reach an endpoint, a rate method may be more practical to obtain a result in a shorter time frame. Some examples of analytes other than enzymes that are measured using rate reaction include ammonia (a waste product of protein metabolism) and amikacin (a therapeutic drug).

CALIBRATION

Calibration is the important process that links the analytical signal with the concentration of analyte.

Calibration uses a series of solutions containing the analyte at known concentrations and observes the signal produced at each concentration. These results can be expressed as a calibration curve. The purpose of a calibration curve is to establish a relationship between the concentration of the analyte and the magnitude of the optical or potentiometric signal given by the measuring device. The relationship can be linear or nonlinear (such as logarithmic or exponential).

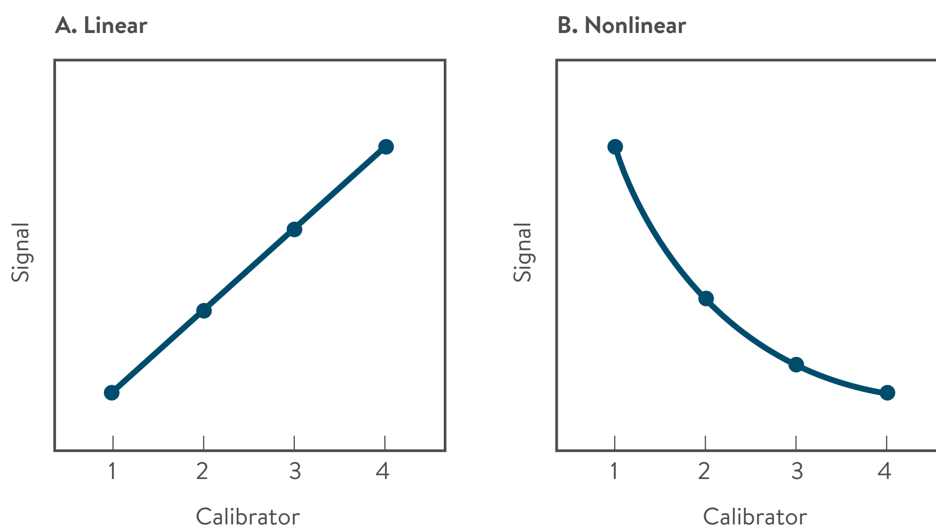


Figure 2-9: Calibration curves.

The calibration curve in Panel A shows the signal rising linearly with increasing concentration of analyte. The curve in Panel B shows the signal falling in a nonlinear fashion with rising analyte concentration. Interpolation (connecting the points on the calibration plot to form the best-fit line or curve) establishes an expected signal for the range of concentrations of analyte that fall between the lowest and highest calibrator. The signal from a sample can be compared to the calibration curve and the concentration of analyte that produces that signal can be determined.

One of the challenges in the calibration process is the determination of the highest and lowest signal that can be reliably measured and related to a concentration of analyte. These limits of measurement are dictated in part by properties of the method and in part by properties of the instrument being used for the test. The laboratory or manufacturer that develops a test method typically determines the analytic measurement range (AMR, also known as the dynamic range) that defines the lowest to highest measurable quantities. Organizations such as the Clinical and Laboratory Standards Institute® (CLSI®; www.clsi.org) publish guidelines for determination of AMR and other characteristics of clinical assays.

Ideally, all samples being analyzed would give signals that are within the AMR. However, for "real-life" clinical assays, this is often not the case. When a signal is outside the AMR, the concentration of analyte in the patient sample cannot be determined with confidence. If the signal is below the AMR, the result is typically reported as less than the lower end of the AMR or less than the lowest calibrator used in the clinical laboratory. If the signal is above the AMR, the result may be reported as greater than the upper limit of the AMR or greater than the highest calibrator used in the lab. Alternatively, the sample may be diluted to bring the analyte concentration within the AMR and reanalyzed. The measured value on the diluted sample is then multiplied by the dilution factor to determine the concentration in the original sample.

Sometimes additional sample can be added to the test reaction to raise the concentration of analyte in the reaction mixture into the AMR. The final result is then corrected for the added volume of sample before reporting.

REVIEW QUESTIONS: SECTION 2

Answers are provided at the end of this Learning Guide.

1. Potentiometric methods are most useful for which of the following types of analytes?

   A. Proteins
   B. Electrolytes
   C. Drugs of abuse
   D. Lipids

2. In a test for albumin, all the albumin reacts very rapidly with an excess of the dye bromocresol purple (BCP) to produce a colored complex. The detector is set to measure the product complex. What method is most suitable for this determination of albumin?

   A. Endpoint (end-up)
   B. Endpoint (end-down)
   C. Rate (rate-up)
   D. Rate (rate-down)

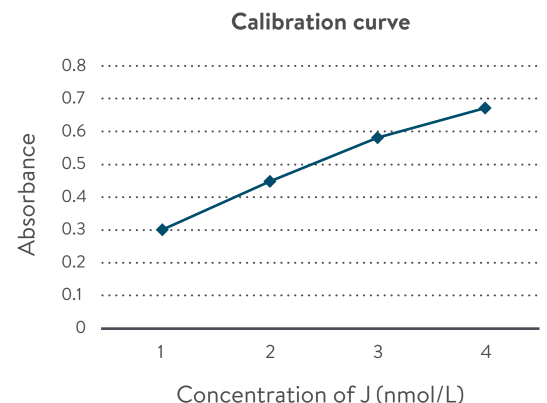
3. Transferrin reacts with a specific antibody to produce immune complexes. What method would be most suitable to measure the concentration of transferrin?

   A. Immunoturbidimetry
   B. Fluorescence
   C. Potentiometry
   D. None of the above

4. What is the best estimate of concentration of substance J in a sample whose absorbance is 0.50?

   A. Between 1 and 2 nmol/L
   B. Between 2 and 3 nmol/L
   C. Between 3 and 4 nmol/L
   D. Greater than 4 nmol/L

Calibrator concentration of J (nmol/L)	Absorbance
1	0.30
2	0.45
3	0.58
4	0.67



SECTION 3

TESTING STRATEGIES TO SELECT FOR A SPECIFIC ANALYTE

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LEARNING OBJECTIVES

After completing this section, you will be able to:

1. Describe some strategies for measurement of a target analyte in complex biologic fluids.
2. Explain how to measure an enzyme analyte or an analyte that is a substrate for an enzyme.
3. Give examples of pretreatment to remove potential interfering substances.
4. Identify examples using antibodies to select for analytes.

OVERVIEW

This section deals with strategies for measuring an analyte when it is present in a complex mixture of biologic molecules. Several approaches are described that are commonly used to select for the target analyte and eliminate or minimize potential interferences from other substances that may be present in the sample.

KEY CONCEPTS

1. Blanking is used to correct for background color in endpoint reactions.
2. The time window chosen for rate reactions can optimize measurement of the target analyte.
3. Enzyme assays, immunoassays and ion-selective electrodes are common approaches to select for a target analyte.
4. Preanalytical separation techniques can be used to isolate the target analyte from interfering compounds.

Measurement of one substance when it is part of a complex mixture of substances provides special challenges. A measurement method that works well for determining the amount of an analyte in a relatively pure form may be completely unsatisfactory when the analyte is in a mixture of cells, proteins, lipids, carbohydrates and trace minerals. Methods for the analysis of analytes in complex biologic mixtures require special approaches to minimize or eliminate interference from other substances. Some of the approaches frequently used in clinical chemistry such as blanking, rate methods, pretreatment, reagent specificity and ion-selective electrodes are described in more detail in the following sections.

The reader should consult Appendix B: References for more detailed information about these topics, especially *Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics*, 7th edition, 2015.

BLANKING FOR ENDPOINT REACTIONS

Blanking is a term that describes a correction for background constituents that contribute directly to the signal being measured. In the case of a colorimetric reaction, blanking measures the innate background color in the sample. Subtraction of the background absorbance from the final absorbance ensures that the background color is not inappropriately attributed to the analyte.

For example, in the measurement of albumin using bromocresol green (BCG), the amount of albumin is calculated from the absorbance of light at wavelength 628 nm — the light absorbed by the green-colored albumin-dye complex. The absorbance is used to compute the amount of albumin present based on a calibration curve (see Section 2 for a review of calibration). However, if other substances in the blood sample also absorb light at 628 nm, their absorbance reading could be incorrectly attributed to albumin and the resultant albumin concentration will appear to be higher than it actually is.

To correct for these other substances, the absorbance of the solution may be measured prior to the addition of the dye and only the change in absorbance above that initial value is used to compute the albumin concentration. Alternatively, the sample may be diluted with a nonreactive solution, such as saline, in a second cuvette and the absorbance of the diluted sample can be used to correct the result.

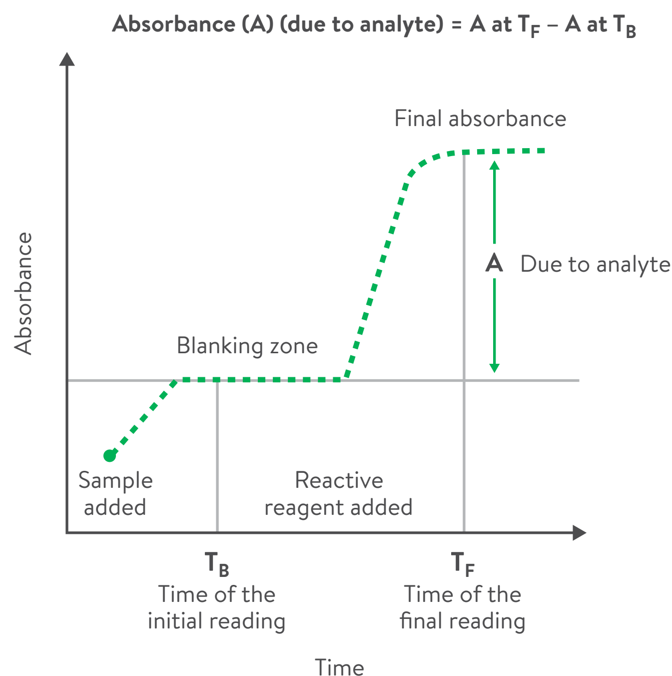


Figure 3-1: Blanking: correction for the contribution of interfering substances by subtracting the signal prior to addition of reactive reagents from the endpoint signal.

Three common interfering substances that are found in plasma and serum are hemoglobin (from red blood cells), lipids (such as triglycerides) that in high concentration result in a turbid (cloudy) solution, and bilirubin (a yellow-orange colored product formed from the breakdown of hemoglobin). These three substances are so commonly found in samples that a special approach is used to assess their presence and correct for their interference in optical analyses. More information on these substances is found in Section 5 under the description of HIL indices.

USE OF SELECTED TIME WINDOWS FOR RATE REACTIONS

Sometimes several substances present in the sample react with the reagents to produce products that absorb light at the same wavelength as the product from the analyte. In such a case, blanking before addition of the reactive reagent will not correct for the interfering substances since the color does not form until the reagent is added. However, in many cases, reaction conditions (such as pH of the solution or concentration of reagents) can be chosen so that the interfering substance reacts at a different time than the target analyte. The interfering substance may react faster and be consumed before the target analyte or may react more slowly and contribute little or no signal in the early time frame of the reaction. If the interferent reacts more rapidly, measurement is taken at time points late in the reaction course when the rate of color change reflects only the target analyte. If the interferent reacts more slowly, measurement is taken at time points early in the reaction when the color change is primarily due to the target analyte.

An example of the value of using a timed window in a rate reaction is seen with the Jaffe method for creatinine. In the Jaffe reaction, creatinine reacts with a solution of alkaline picrate to form a red-orange product. Unfortunately, many other substances found in biologic samples also react with alkaline picrate to form red-orange products. Some of these include acetoacetate and protein. It was found that acetoacetate reacts completely within the first 20 seconds and protein demonstrates a lag time, reacting only after 1 or 2 minutes. So a time window that begins sometime after 20 seconds and ends within the first minute will reflect product formed from creatinine with little interference from either acetoacetate or protein.

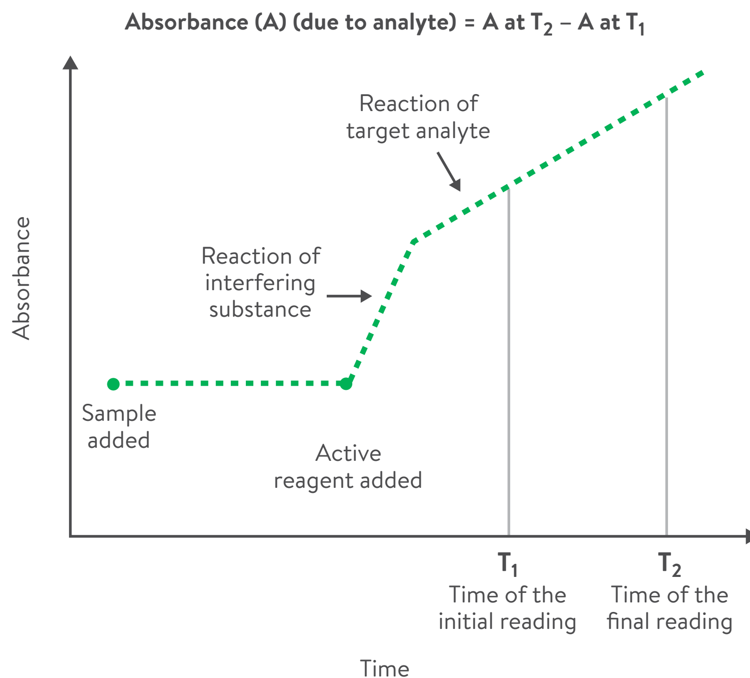


Figure 3-2: Rate reaction with measured times chosen to reflect target analyte.

PRETREATMENT

Sometimes it is possible to treat the sample prior to analysis to physically or chemically remove potential interfering substances. Pretreatment may be done "offline" or "online." Pretreatment performed "offline" means that the treatment is done in a manual step before the sample is loaded on an automated analyzer or placed in the reaction cuvette for analysis. Pretreatment performed "online" means that the treatment is automated on the analyzer and is carried out as part of the total analytical process, usually in the same reaction cuvette that is used for the measurement step.

Measurement of high density lipoprotein (HDL) cholesterol, typically about 25% of the total cholesterol in serum, requires removal of all non-HDL cholesterol such as low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol prior to the measurement step. The pretreatment can be done offline or online.

OFFLINE PRETREATMENT

Offline pretreatment involves mixing the serum with an agent such as polyethylene glycol (PEG) that reacts with the non-HDL cholesterol particles. This step is offline pretreatment because it is carried out manually and not automatically on an analyzer. A sample of serum is mixed with PEG and a precipitate containing the LDL and VLDL particles is formed. The precipitate can be forced to the bottom of the tube by centrifugation, leaving a clear solution that contains the HDL. This clear solution is used as the sample for HDL cholesterol. The test involves treatment with a reagent specific for cholesterol, such as cholesterol esterase, that produces a product that can be measured photometrically.

ONLINE PRETREATMENT

An online approach involves a two-step reagent treatment of the sample in the reaction cuvette. The first step introduces a reagent (cholesterol oxidase) that selectively destroys the non-HDL cholesterol that is not bound to lipoprotein, leaving only HDL cholesterol in solution. The second step detects the remaining HDL cholesterol by its reaction with a reagent specific for cholesterol, such as cholesterol esterase, to produce a product that can be measured photometrically.

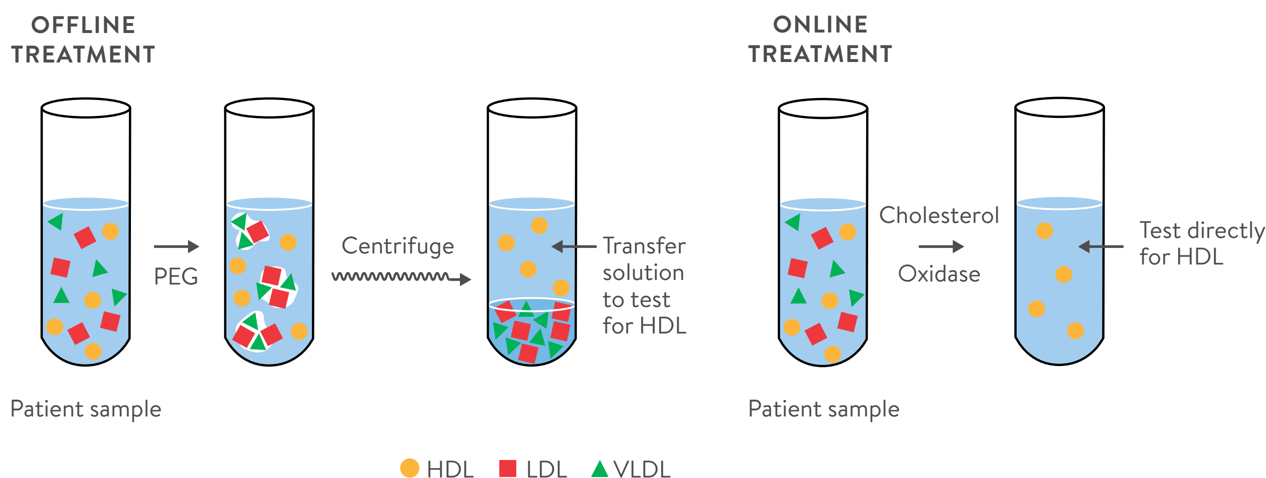


Figure 3-3: Measurement of HDL.

CHOOSING METHODS THAT ARE HIGHLY SELECTIVE

ENZYMES

Enzymes are biochemical catalysts (substances that increase the rate of a biochemical reaction without being consumed in the reaction). They are often exquisitely selective for one and only one chemical structure. A chemical structure that is specifically acted on by the enzyme is called a substrate. Enzymatic reactions can be used for determination of either a substrate concentration or an enzyme's activity.

> An enzyme is a biochemical catalyst, a substance that increases the rate of a reaction without being consumed in the reaction. Each enzyme catalyzes conversion of a specific molecule, referred to as the substrate.

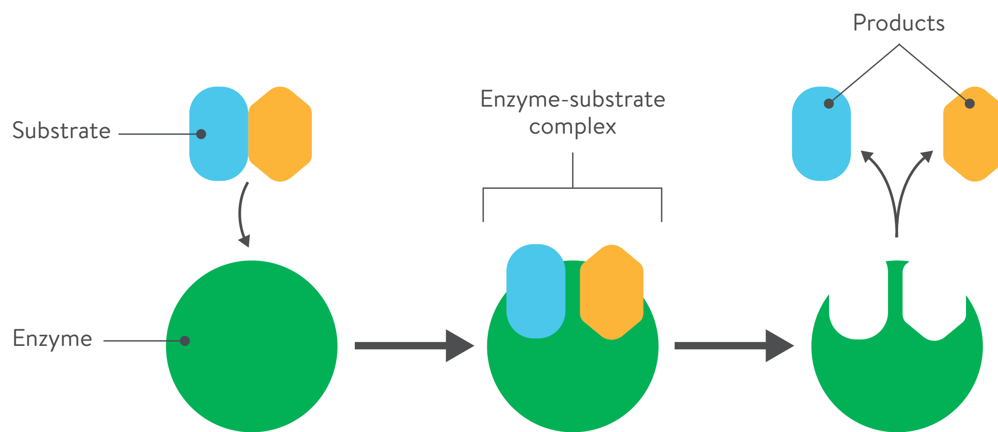


Figure 3-4: Enzyme cleaves substrate to produce photometrically measurable product.

DETECTION OF SUBSTRATES

For many analytes in biologic fluids, such as glucose, cholesterol, bilirubin or creatinine, nature has provided enzymes that selectively react with these important molecules. Such enzymes can be used to catalyze the conversion of these molecules (substrates) in reactions that generate products that can be observed photometrically. For example, glucose can be converted by the enzyme hexokinase to glucose-6-phosphate, which in turn can be used to produce a molecule of nicotinamide adenine dinucleotide phosphate (NADPH). For each molecule of glucose, one molecule of NADPH is produced. NADPH can be measured in the ultraviolet region at 340 nm. Detection of a biologic substrate like glucose can be carried out as an endpoint reaction by measuring the maximum amount of NADPH formed or as a rate reaction by measuring the rate at which NADPH is formed.

DETECTION OF ENZYMES

Determination of enzymatic activity requires a rate reaction. Many analytes of interest are themselves enzymes. (See Sections 1 and 6 for more information about enzymes measured in the clinical laboratory.) To measure the amount of enzyme present, a substrate that is recognized only by that enzyme is used. For example, the enzyme lipase releases fatty acids from triglycerides and diglycerides. Lipase activity is measured by using products from lipase action on diglycerides to generate glycerol molecules. These glycerol molecules produce a colored product that absorbs light at 548 nm. The rate of increase in absorbance at 548 nm is a function of lipase activity. Enzymatic reactions can be coupled and occur in multiple steps within a chemistry test.

PANCREATIC LIPASE	1,2-diglyceride + H_2O	→	2-monoglyceride + fatty acid
MGLP	2-monoglyceride + H_2O	→	glycerol + fatty acid
GK	glycerol + ATP	→	glycerol-3-phosphate + ADP
GPO	glycerol-3-phosphate + O_2	→	dihydroxyacetone phosphate + H_2O_2
POD	$2H_2O_2$ + 4-aminoantipyrine + TOOS	→	quinone dye + H_2O

ANTIBODIES

Antibodies (immunoglobulins) are formed by the immune system in direct response to "foreign" substances called antigens. Deliberate exposure of an animal to an antigen (immunization) generates antibodies that are specific for that antigen. The antigens may be analytes like proteins (e.g., transferrin) or drugs (e.g., amikacin). The antibodies produced by such immunizations are termed "anti-(analyte name)" antibodies. For example, antibodies produced by a goat against a human transferrin protein are called goat anti-human transferrin antibodies. Antibodies produced in a rabbit against the drug amikacin are called rabbit anti-amikacin antibodies. These antibodies can be used to selectively measure transferrin or amikacin in a human serum sample. Examples of formats for assays using antibodies (immunoassays) include the following.

IMMUNOPRECIPITATION (IMMUNE COMPLEXES)

Antibodies to protein antigens can bind to multiple sites (or epitopes) on the protein molecule and can cross-link many different molecules of the same protein to form an insoluble precipitate composed solely of antibody and antigen molecules. This immunoprecipitate can be detected using a turbidimetric method. For example, the protein transferrin can be mixed with antitransferrin antibodies and the resultant immunoprecipitate can be quantified in a turbidimetric rate or endpoint reaction.

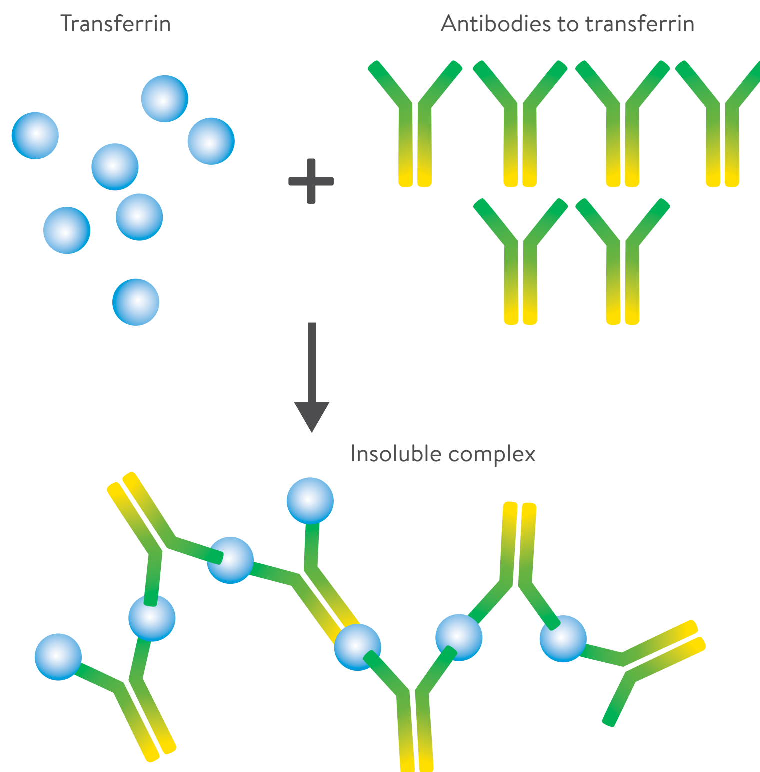
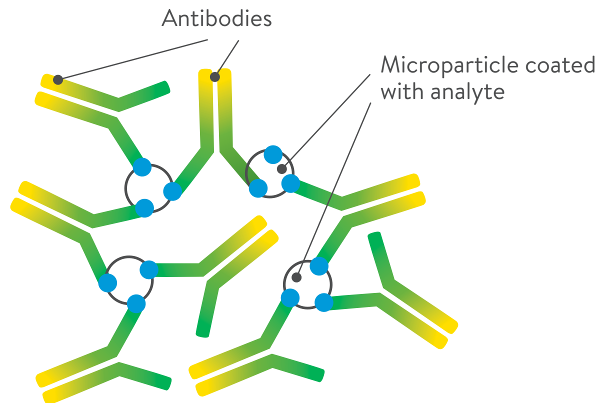


Figure 3-5: Immunoprecipitation of transferrin — formation of large insoluble complexes of cross-linked antibodies and transferrin.

PARTICLE-ENHANCED TURBIDIMETRIC INHIBITION IMMUNOASSAY (PETINIA) AND PARTICLE-ENHANCED TURBIDIMETRIC IMMUNOASSAY (PETIA)

When an analyte is a small molecule that cannot be cross-linked to produce an immunoprecipitate, it is still possible to use a turbidimetric method. A microparticle is coated with the analyte. When suspended in solution, the microparticle is too small to affect light passing through the solution, but when antibodies to the antigen are added, the particles aggregate into larger complexes that prevent passage of light and can be measured by turbidimetry. When antigen is present in a patient sample, it competes for the antibodies and prevents aggregation. For example, if the drug amikacin is coated on microparticles and these amikacin particles are mixed with a serum sample containing amikacin, the turbidimetric signal upon addition of anti-amikacin antibodies will be much smaller than when no drug is competing for the antibodies. The decrease in rate of formation of light-blocking particles can be correlated with the amount of amikacin in the serum sample. In a variation of this technique, microparticles may be coated with antibodies to an analyte, perhaps a protein such as β_2 microglobulin (B2M). If the analyte is bound by the antibodies on the microparticles, large immunocomplexes can be formed that will scatter light. An increase in the scattered light correlates to the concentration of analyte in the patient specimen.

In absence of a drug in a sample solution, particles are extensively cross-linked by antibodies.



The presence of a drug in a sample solution can be detected by the decrease in turbidity due to fewer cross-linked particles.

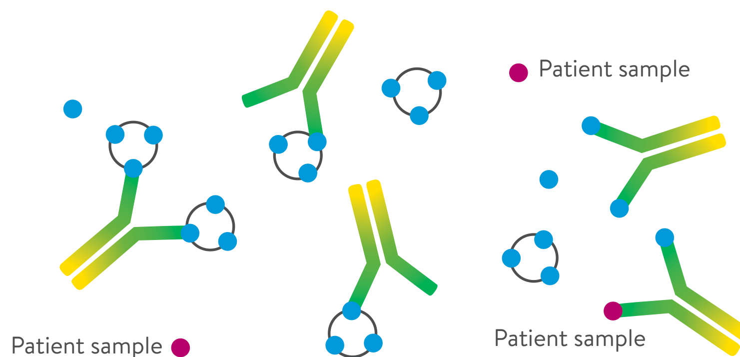


Figure 3-6: Particle-enhanced turbidimetric immunoassay (PETIA).

ENZYME-MULTIPLIED IMMUNOASSAY TECHNIQUE (EMIT®)

In this format, an analyte of interest in serum competes with a modified version of that same compound for antibodies to the analyte. The modified version is linked to an enzyme. The enzyme is active unless the attached analyte is bound to antibodies. For example, theophylline linked to the enzyme glucose-6-phosphate dehydrogenase generates a product called nicotinamide adenine dinucleotide phosphate (NADPH) that can be measured spectrophotometrically. When the theophylline-enzyme complex is bound to anti-theophylline antibodies, no enzymatic reaction occurs and no NADPH is produced. If the drug theophylline is present in serum, it can bind to the anti-theophylline antibodies and displace the enzyme-theophylline complex, allowing the enzyme, whose active site is no longer blocked by the antibody, to produce NADPH, which is measured optically in the UV region at 340 nm. The more theophylline in the serum sample, the greater the amount of free enzyme-theophylline complex and the more NADPH produced.

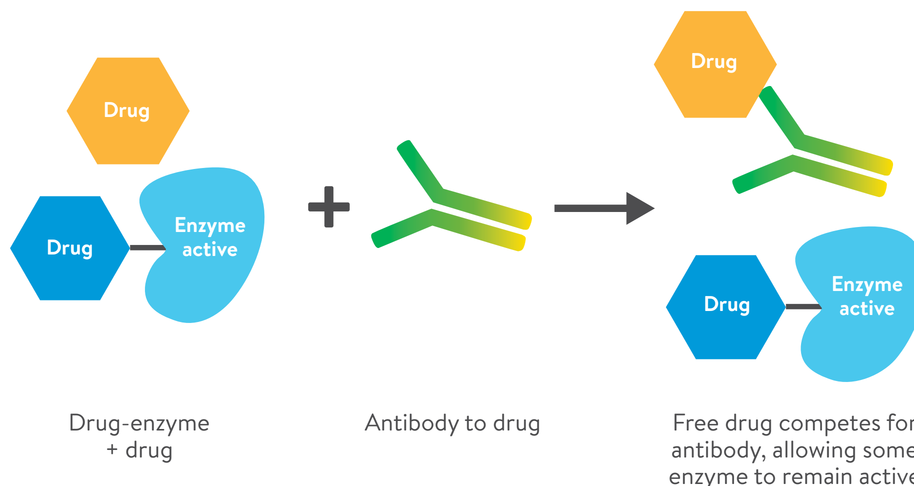


Figure 3-7: Enzyme-multiplied immunoassay technique (EMIT®).

ION-SELECTIVE ELECTRODES

Specially designed ion-selective electrodes (ISEs) allow potentiometric measurement of a single type of ion without interference from other ions. Ions that can be measured in this way include sodium (Na^+), potassium (K^+), chloride (Cl^-), lithium (Li^+), calcium (Ca^{++}) and magnesium (Mg^{++}). Although typical electrodes like those in batteries or in chemical cells can react with many different kinds of ions, ISEs use membranes with very selective permeability or sensitivity to the analyte ion's size and charge. The membranes may have pores that limit the size of ions that may enter the membrane. They may be impregnated with chemicals, called ionophores, which allow only selected ions to be sensed by the electrode. For example, a polymer membrane that incorporates an ionophore called valinomycin is highly selective for potassium with little to no response to physiologic concentrations of other ions like sodium, calcium or magnesium. An ion-selective electrode of this design is ideal for measurement of potassium ions in the presence of physiologic concentrations of sodium ions with no bias caused by the larger number of sodium ions.

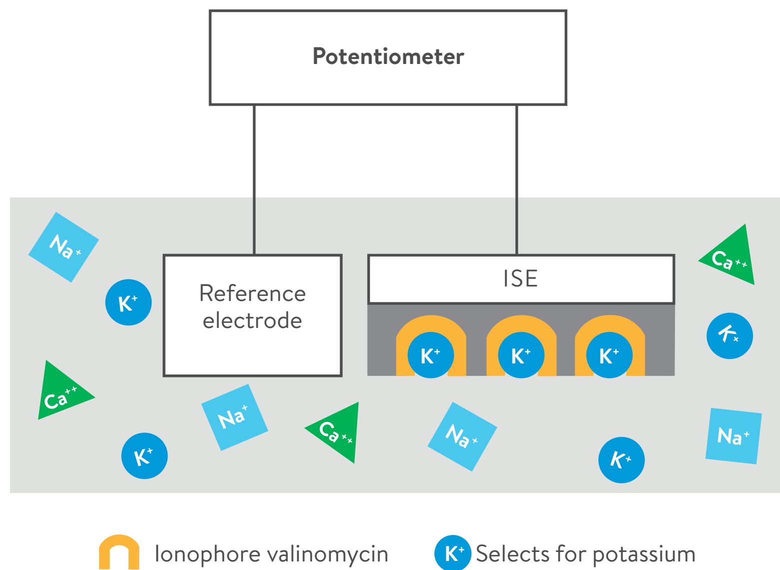


Figure 3-8: Ion-selective electrode.

REVIEW QUESTIONS: SECTION 3

Answers are provided at the end of this Learning Guide.

1. When a serum sample has intrinsic color that absorbs at the same wavelength used to detect the reaction product, what technique could help distinguish the color produced by the analyte from the intrinsic color of the sample?

    - A Blanking
    - B Immunoturbidimetry
    - C Ion-selective electrode
    - D PETINIA

2. Which of the following analyses would best be done using a photometric rate reaction?

    - A Measurement of lipase activity
    - B Determination of albumin with the dye bromocresol green
    - C Determination of potassium in the presence of excess sodium
    - D None of these could be done using a rate reaction

3. Pretreatment is designed to do which of the following?

    - A Ensure the concentration of analyte is in the measurable range
    - B Remove substances that could be erroneously measured as analyte
    - C Adjust the wavelength of light used for analysis
    - D Introduce a fluorophore

4. Which of the following would not be a typical methodology for a clinical chemistry test?

    - A Immunoturbidimetry
    - B Microscopy
    - C EMIT®
    - D ISE

SECTION 4

ACCURACY

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LEARNING OBJECTIVES

After completing this section, you will be able to:

1. Distinguish between precision and accuracy.
2. Describe how calibrator values are assigned.
3. Identify the roles of proficiency testing (PT)/external quality assurance (EQA) and quality control testing programs in ensuring the accuracy of test results.

OVERVIEW

This section reviews procedures used to establish the accuracy of test results and introduces some statistical concepts that describe how close a measurement is to the true value.

KEY CONCEPTS

1. Laboratory tests must meet precision and accuracy standards.
2. Accuracy, closeness to a true value, depends on a valid calibration process.
3. Calibrator value assignment is linked to a certified reference material, a recognized reference method or a consensus process that provides "traceability."
4. Laboratories use quality control and proficiency testing to monitor the precision and accuracy of test methods.

This section addresses the concept of accuracy and describes procedures that manufacturers and clinical laboratories use to ensure that a result reported for a test is a value that truly reflects the amount of analyte present in the sample.

The reader should consult Appendix B: References for more detailed information about these topics, especially *Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics*, 7th edition, 2015; the Lab Tests Online® website (www.labtestsonline.org); and the standardization program websites (www.cdc.gov/labstandards/crmln.html; www.ngsp.org; and www.ifcchba1c.net).

PRECISION AND ACCURACY

If the true value for an analyte is represented by the bull's-eye on a target, then accuracy is the equivalent of hitting that bull's-eye every time a measurement is made. Inaccuracy is the opposite and means missing the target or bias.

Accuracy of analytic methods can be described using the concepts of precision and bias. Precision is the reproducibility of a measurement. Imprecision is the opposite, meaning increased variability in the measurement. Bias is the deviation from the true value (also known as the target value). **Figure 4-1** shows the relationship between precision and accuracy.

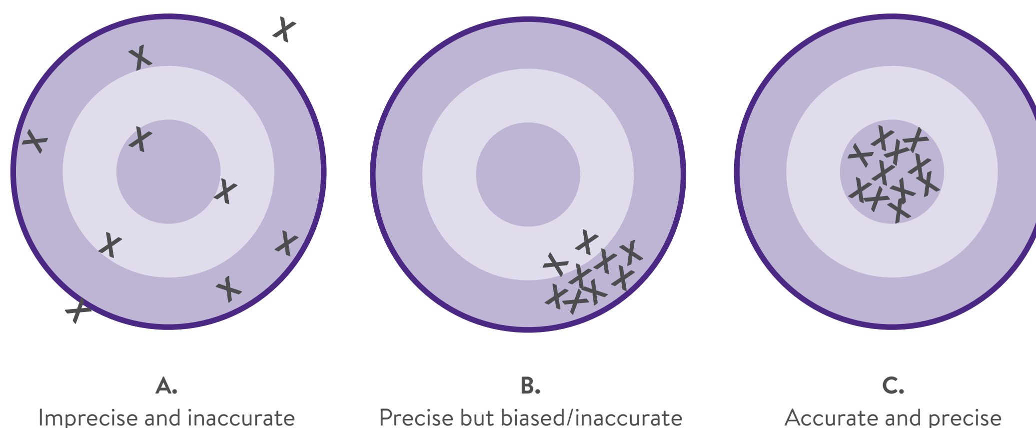

Figure 4-1: Precision and accuracy.

If a sample is divided into several aliquots (a laboratory term for splitting a sample into a smaller portion) and each aliquot is tested for the amount of analyte in it, the results should ideally be the same for all aliquots. This is rarely the case because of the inherent variability of any test method. The closer the values are to each other, the more precise (reproducible) the method. Panel A represents imprecision with values that are widely scattered. In Panels B and C, the methods are equally precise (reproducible), but the results in Panel B are far from the true value (biased), while the results in Panel C are close to the true value (accurate) and precise (reproducible).

PRECISION

Precision is typically a function of the analytic method that is being used. Precision reflects the innate reproducibility of the signal generated by the test solution and the stability of the analyzer used to measure that signal.

> Standard deviation (SD) is a measure of precision. It reflects the spread of values from the mean (average) value.

> Coefficient of variation (%CV) is the standard deviation expressed as a percent of the mean.
>
> **CV = (SD/Mean) × 100**

Precision is described quantitatively by the standard deviation (SD). Standard deviation is calculated using the mean value (average) of all test values and the deviations of each measurement from the mean. The standard deviation reflects the spread of values and is often represented by the symbol SD or σ. A test with a mean of 10 and SD of 1 is more precise than a test with a mean of 10 and SD of 4. The mean and SD can be expressed as 10 ± 1 (where 10 is the mean and 1 is the standard deviation). Precision can also be expressed as a percent of the mean using the following formula: 100 × (SD/mean). In the example, the standard deviation is 10% of the mean. When precision is expressed as percent of the mean, the value is termed coefficient of variation (%CV).

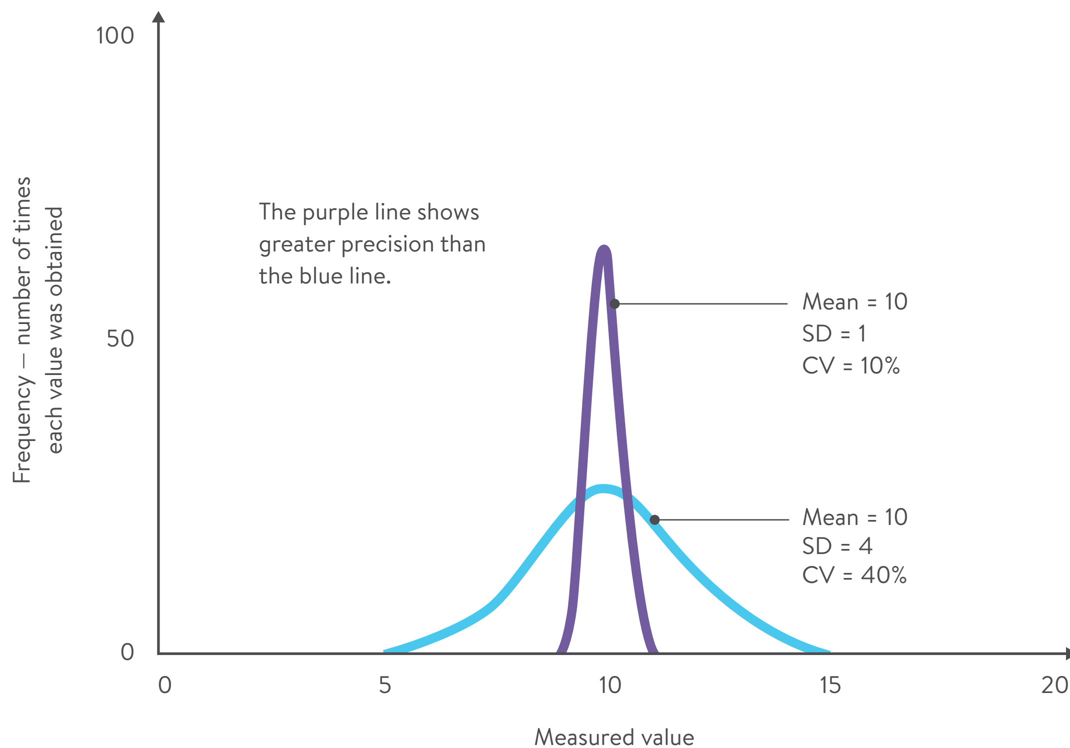

Figure 4-2: Graphic representation of precision of a repeated measurement.

BIAS

Bias is typically a function of the calibration process. Calibration is the step that links the magnitude of an optical, electrochemical or any analytical signal to a specific amount of analyte. The accuracy of the calibration process is dependent on the values that are assigned to the calibrators.

Bias is usually described as a percent reflecting the difference between the measured value and the true value. For example, if the target value is 50 and the measured value is 45, the bias is 5 parts out of 50 or 10% ([5/50] × 100).

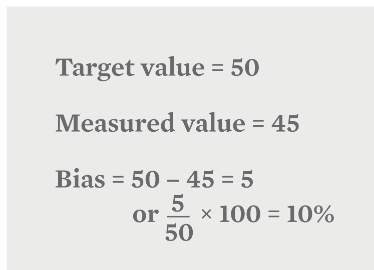

Figure 4-3: Bias = deviation from target value.

ASSIGNING VALUES TO CALIBRATORS

Because getting the right answer, or the "true value," is dependent on the correct interpretation of the analytical signal, the validity of the calibration curve is a critical component of test accuracy. Assignment of values to calibrators relies on a process that links the value to some agreed upon standard material or reference method. This is termed "traceability." Two typical approaches to calibrator preparation are (1) use of primary reference materials and methods or (2) use of consensus methods and materials.

USE OF PRIMARY REFERENCE MATERIALS AND METHODS

If an analyte can be isolated in a pure, well-defined form, the use of primary materials and methods is usually chosen as the basis for calibration. Assignment of values to calibrators begins with identification of the substance that will serve as the "gold standard" for an analyte. For a simple substance such as calcium, a particular form is chosen to be the primary reference material, perhaps calcium carbonate or calcium phosphate. Then the actual amount of calcium in the chosen material is determined. This determination is performed using a primary or definitive reference method, such as atomic absorption in the case of calcium.

The preparation of primary reference materials and assignment of reference values to them are specialized activities. Reference materials are typically prepared by government agencies or professional organizations such as:

- National Institute of Standards and Technology (NIST®)
- American National Standards Institute® (ANSI®)
- World Health Organization (WHO)
- Clinical and Laboratory Standards Institute® (CLSI® — formerly NCCLS)
- International Federation of Clinical Chemistry (IFCC)
- Institute for Reference Materials and Methods (IRMM)
- National Institute for Biological Standards and Control (NIBSC)
- The Joint Committee for Traceability in Laboratory Medicine (JCTLM) maintains a database of reference materials, reference methods, and laboratories that maintain reference methods; the database may be accessed and searched at www.bipm.org/en/committees/jc/jctlm

See Appendix B: References for links to these organizations.

Primary reference materials are too costly and often not suitable to be used as calibrators in clinical laboratories. They may be insoluble in biological matrices (body fluid samples), or they may be in chemical forms that differ from those present in biological samples, and therefore unable to be detected by the methods used in a clinical laboratory.

Secondary reference materials, or materials that are more suitable for analysis by typical clinical laboratory methods, are used instead. Their values are assigned by comparison with the primary reference materials using an analytic method that is robust enough to measure and compare the analyte in both the primary and secondary materials. The primary reference material serves as the calibrator to assign a value to the secondary material. Secondary reference materials are prepared in a matrix (solution) that resembles the actual patient specimens (e.g., serum, plasma, urine). These materials are commutable, that is, provide an analytical response similar to that of an actual patient specimen. Commutability can be confirmed by testing reference materials and fresh patient specimens together using two or more routine (field) methods. If the reference material is commutable, the results from the field methods should recover the target values assigned by a reference method and the analytical response should be consistent with that of the fresh patient specimens.

Calibrators for clinical lab tests are often prepared in a solution that resembles a patient sample (e.g., blood, serum). The value of an analyte in the calibrator solution is established by comparison to a secondary reference material. This comparison and assignment of the calibrator value is done by the manufacturer of the reagents and test equipment.

The primary reference (gold standard) material serves as the calibrator to assign a value to the secondary reference material, which in turn is used to assign calibrator values for use within the laboratory. This unbroken chain of connections is termed “traceability.” Each calibrator value is traceable to some identified standard material. **Figure 4-4** illustrates the traceability chain for cholesterol as standardized by the Cholesterol Reference Method Laboratory Network (CRMLN), an international network of laboratories that provides certification to manufacturers of equipment and reagents for measurement of cholesterol.

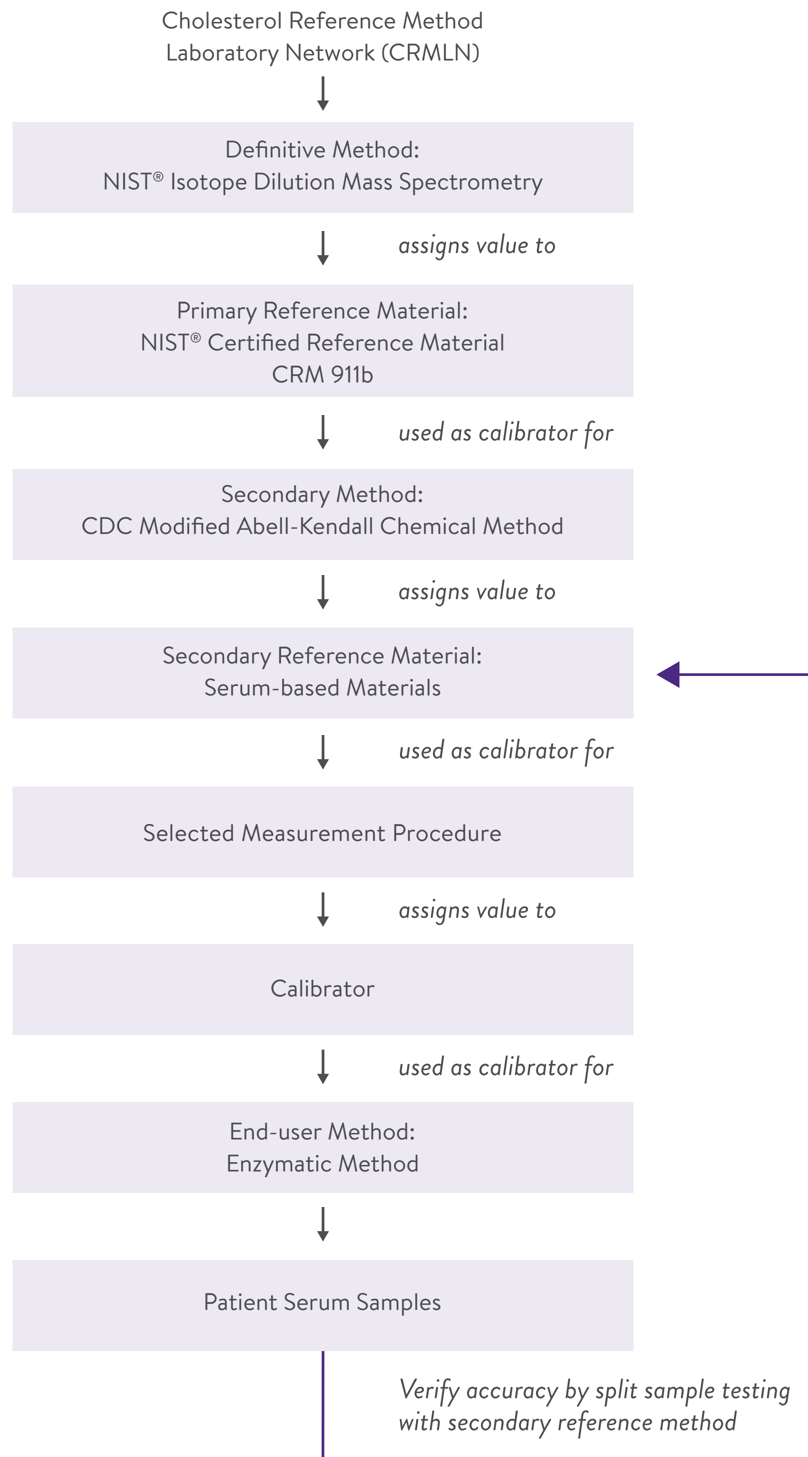

Figure 4-4: Traceability to primary reference material.

USE OF CONSENSUS VALUES

For many analytes, the preparation of a pure reference material that can serve as a primary standard is impossible. Analytes such as proteins often have many different forms that may be present in differing amounts in different patients. Thus, it is difficult to identify one form of the protein as an ideal reference material. Other analytes, like bilirubin, are inherently unstable and break down when exposed to light or air or when separated from other stabilizing molecules in solution. Enzymes often lose their enzymatic activity if isolated in pure form. For these types of analytes, no suitable primary reference material can be prepared. Instead, values for these analytes are traceable to a consensus value based on an approach that has been established by agreement among laboratory professionals.

Hemoglobin (HbA1c), the most important test for long-term diabetic control, is an example of a test that is standardized by a consensus process. When hemoglobin is exposed to glucose, it can undergo a modification in which a glucose molecule chemically attaches to the protein. The product is called a glycohemoglobin (or glycated hemoglobin). Since this attachment can occur at any of several different sites on the hemoglobin molecule, the result is a heterogeneous mixture of unmodified hemoglobin and various glycohemoglobin molecules.

Values assigned to the test for HbA1c are based on a method that was used in a large clinical trial called the Diabetes Control and Complications Trial (DCCT). That trial identified target values for HbA1c to achieve optimum control of diabetes. Since clinical interpretation of the test result is based on the outcomes from that trial, the clinical utility of a patient test result is tied to how well it matches the results from the method used in that trial. That method, a high-performance liquid chromatography (HPLC) method using Bionix® resin, has been adopted as the consensus method by the National Glycohemoglobin Standardization Program (NGSP).

Figure 4-5 illustrates how results from methods used by clinical laboratories are traceable to the values obtained by the consensus method from a NGSP laboratory. The consensus method is used to calibrate secondary methods in special certified laboratories. Manufacturers as well as clinical laboratories can compare the results from their method with the results from a secondary lab to confirm accuracy. Manufacturers use these results to assign appropriate values to calibrators so patient samples will give results comparable to the consensus method. All comparisons are carried out on blood samples collected from diabetic and nondiabetic donors.

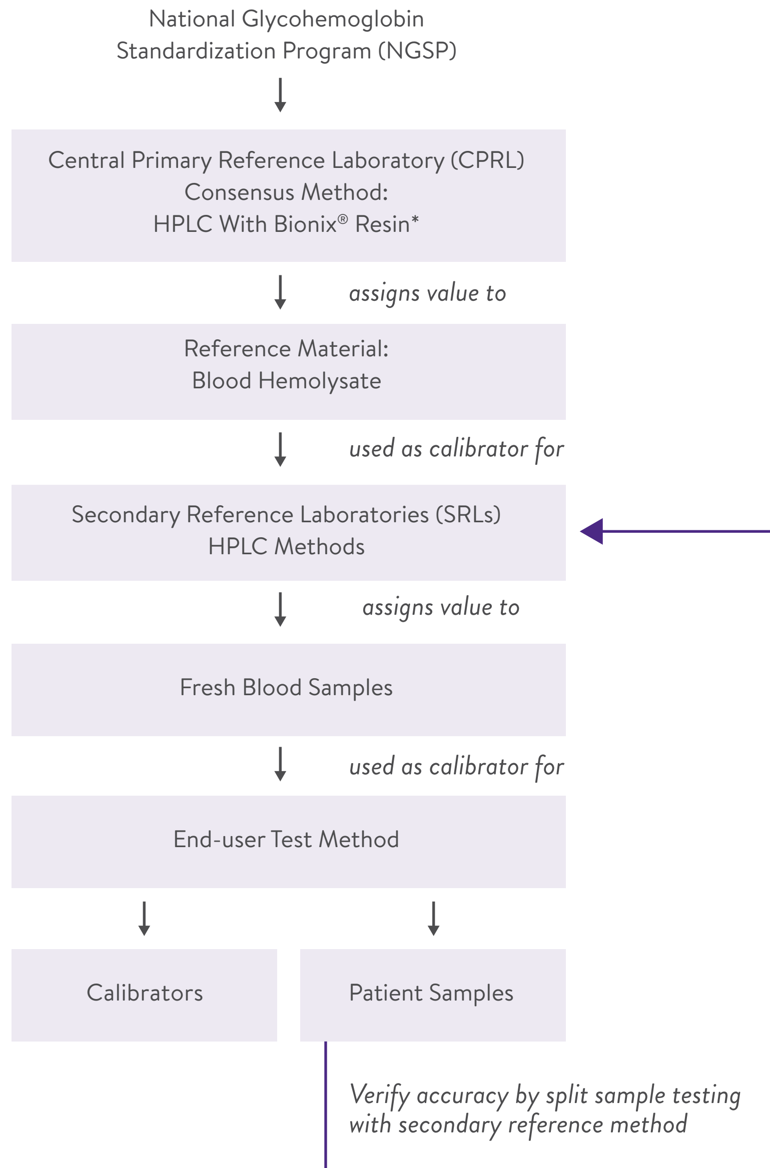

Figure 4-5: Consensus values.

*Bionix resin high-performance liquid chromatography (HPLC) was the method used in the Diabetes Control and Complications Trial (DCCT) that identified target HbA1c levels for good diabetic control. This standardization system provides traceability to the interpretation scheme recommended for treatment of diabetes based on that research trial.

Note: An alternative reference measurement system for standardization of HbA1c has been adopted by the International Federation for Clinical Chemistry (IFCC). In this approach, two purified forms of hemoglobin are isolated and used for calibration. One is unmodified and carries no glucose molecules. The other carries a single glucose molecule attached to the amino acid valine at the amino end of one of the beta chains of hemoglobin. A series of standard solutions is prepared by mixing different proportions of these two forms of hemoglobin. The results are expressed as a ratio of the modified form to the unmodified form. In addition, the IFCC has developed two equivalent reference methods: Liquid Chromatography/Capillary Electrophoresis and Liquid Chromatography Mass Spectrometry. Results from the IFCC and NGSP methods can be interconverted using a validated master equation. The NGSP and IFCC work with each other to ensure continuous standardization of HbA1c methods, interconversion of results between % HbA1c and mmol/mol HbA1c, and clinically acceptable performance of commercial test methods.

MATRIX EFFECTS

Many clinical laboratory methods are affected by the sample matrix — a term for the physiologic or artificial milieu that contains the analyte. Sometimes two different methods that give the same result for a patient sample may give different results when analyzing synthetic samples such as calibrator fluids, proficiency testing (PT) samples and quality control (QC) samples. Ideally, these synthetic samples should mimic a patient sample, but many times they do not because the matrix has undergone a type of manufacturing process and does not resemble a fresh human patient specimen. The manufacturing process stabilizes and extends the analyte life during shipping and storage, but this process changes the matrix of the native human sample.

Calibrator, PT and QC solutions differ from patient samples in that they are supplemented with many different substances to generate wide ranges of analyte concentrations. In addition, these samples are often frozen or lyophilized (freeze-dried to remove all liquid) to minimize decomposition of analytes during storage. The process of freezing or lyophilization followed by thawing or reconstitution with liquid may also change some properties of the solution. When the addition of extra substances, freezing or lyophilization alters the solution properties in a way that biases the measured result, the bias is said to result from a "matrix effect."

When the sample matrix (a term for the physiologic or artificial milieu that contains the analyte) contributes to a bias of the measured value, the bias is said to result from a "matrix effect."

Calibrator values are assigned for a specific method and instrument. If calibrators are used with a different method, matrix effects may result in inaccurate calibration. In PT programs, different methods may need to be evaluated using method-specific target values reflecting varied influences of the PT material matrix. The results are separated into peer groups of participants who use the same method, the same reagent or the same analyzer.

ENSURING ACCURACY

Clinical laboratory testing must meet numerous scientific and regulatory criteria before it is acceptable for medical decision-making. The responsibility for meeting these criteria is shared by the manufacturer and the clinical laboratory.

A desirable goal for accuracy is that the combined imprecision and bias should not exceed the typical within-subject biologic variation (the natural biologic fluctuations of the analyte within an individual over time). This ensures that any changes greater than typical biologic variability in a patient's test results reflect real changes in the patient's condition rather than laboratory variability.

The biologic variation is taken into account when setting the "error budget" or total error allowable (TEa) for a laboratory test. The TEa is the amount a test result may deviate from the "true value" and still be acceptable.

The TEa has two components: the bias from the true value and the imprecision of the test.

$$\mathbf{TEa = Bias + (2 \times CV)}$$

For cholesterol, the within-subject biologic variability* predicts that 95% of test values for an individual would fall within ± 10.8% of the true value. The total error budget for a cholesterol test should, therefore, be less than 10.8%. The National Cholesterol Education Program (NCEP) requires an error budget for cholesterol certification that is less than 9%. Thus, for NCEP certification, a method with a CV of 3% could have a bias up to 3% without exceeding the error budget.

$$\mathbf{TEa = 3\% + (2 \times 3\%) = 9\%}$$

MANUFACTURER RESPONSIBILITIES

Before test methods are introduced in the clinical laboratory, the manufacturer must ensure that the test's performance criteria fall within acceptable TEa targets. The method should be optimized to provide suitable reproducibility and bias, and the calibration process should include traceability to ensure that an accurate value is assigned to the test result.

In the United States, the Clinical Laboratory Improvement Amendments (CLIA) sets the target ranges for total allowable error for many common clinical chemistry tests. In Germany, the Guidelines for Quality Assurance of Medical Laboratory Examinations of the German Medical Association (RiliBÄK) define the acceptable limits. Other professional organizations or regulatory authorities may also set performance targets for many analytes. Manufacturers must ensure that methods are capable of meeting these targets.

*More information about biologic variability and target values for accuracy ranges can be found at www.westgard.com/guest17.htm.

Examples of TEa targets for some selected analytes

Analyte	CLIA acceptable range*	RiliBÄK acceptable range**
Albumin	± 10%	± 12.5%
Chloride	± 5%	± 4.5%
Cholesterol	± 10%	± 7%
Glucose	± 10% or 6 mg/dL (whichever is greater)	± 11%
Total protein	± 10%	± 6%
Triglycerides	± 25%	± 9%
Alcohol, blood	± 25%	± 15%
Theophylline	± 25%	± 13%

*U.S. Code of Federal Regulations (42 CFR 493.931).
**German RiliBÄK guidelines.

CLINICAL LABORATORY RESPONSIBILITIES

Clinical laboratories must demonstrate that they can perform a test and get acceptable results using the method in day-to-day operations. Two systems are used to ensure that clinical laboratories are performing acceptably. These are quality control testing and proficiency testing.

QUALITY CONTROL (QC) TESTING

In QC testing of the same sample, a control sample is analyzed multiple times (usually daily) over an extended period of time (weeks to months) to ensure that the test gives reproducible results. The QC results are plotted on graphs called Levey-Jennings plots. These plots show results for the analysis of the QC sample compared to the expected value and acceptable range of values. The target value and range of values are determined by the laboratory for each QC sample.

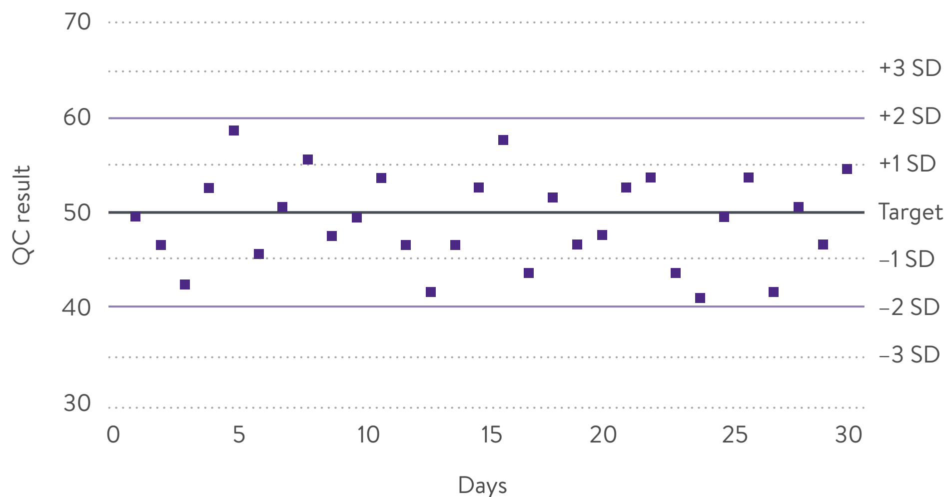

Figure 4-6: Levey-Jennings graph showing acceptable reproducibility over 1 month.

Typically, two QC samples are monitored for every test. One QC sample is termed "normal" and has a target value that falls within the expected reference interval for that test. The second QC sample is termed "abnormal" and has a target value that is outside the reference interval in a direction that is clinically significant. When appropriate, the abnormal QC sample will have a concentration that is important for clinical decision-making. For example, fasting glucose in a nondiabetic is expected to be between 70 and 100 mg/dL (3.9 and 5.6 mmol/L) and the diagnosis of diabetes is made when fasting glucose is above 126 mg/dL (7.0 mmol/L). Two QC samples might be chosen at levels of 85 mg/dL (4.7 mmol/L) for the "normal" QC sample and 145 mg/dL (8.0 mmol/L) for the "abnormal" QC sample. If the laboratory deals with patients having low glucose (hypoglycemia), it may also include a third QC sample at a low value such as 36 mg/dL (2.0 mmol/L).

The purpose of the QC sample is to monitor the reliability of the test. When the daily QC results fall within the range of acceptable values and are scattered symmetrically around the target value, the method is said to be "in control." When results fall outside the target window or show a drift away from the target value over time, the QC results indicate a problem. There are a number of rules that are used to assess the QC patterns and determine when to take action. Usually action is taken when the number of results that fall outside of the 2 SD range exceeds expectation or when a series of consecutive results over many days falls on one side of the target value or drifts away from the target value.

When the QC results show a problem, a systematic investigation into the source of the problem is required. The problem may rest with the integrity of the QC sample itself, the stability of the reagents for the test method, the calibration curve or an instrument problem in a component such as the light source, the sampling system, the mixing system or temperature control. The problem could also lie with the operator and be due to a procedural error or staff technique. Before a patient sample can be tested and results reported, the source of the problem needs to be identified and corrected, and the analytic system brought back "in control."

Taking prompt action as soon as the QC system indicates a potential problem ensures that the laboratory does not report inaccurate test results on patient samples.

PROFICIENCY TESTING (PT)

In proficiency testing, also known as external quality assurance (EQA), an external organization (professional organization or governmental agency) sends sets of unknown samples (called PT samples or challenges) to the laboratory for analysis. These samples must be treated and tested as if they were patient samples, and the results must be submitted to the PT provider for review.

The PT provider usually sends two to five unknown samples for each analyte and sends sets of samples for all analytes several times per year.

The PT provider evaluates the results from many different laboratories and grades the performance based on how close each laboratory's results are to the target values for each analyte. Acceptable results must fall within the accuracy ranges established for the test. In the United States, many accuracy ranges are defined by CLIA. If there is no established accuracy range for an analyte, the test result is compared with the results from other laboratories that tested the same samples. A statistical range is established for all laboratories reporting results for that analyte or sometimes for subsets of laboratories using the same method (termed peer groups). An alternate, and more demanding, approach to PT is "accuracy-based grading." The PT samples are assigned target values by using reference materials or methods, and laboratories must recover the target values within predetermined acceptance limits to pass the PT survey challenge. In this approach, the acceptance limits are the same for all laboratories and methods, as opposed to peer group grading.

If a laboratory fails to perform satisfactorily, the laboratory may be prohibited from performing patient tests for the failed analytes until it can identify and correct the problem and successfully analyze a new set of PT samples from the provider.

Only when a laboratory meets the performance criteria for accuracy can it report test results for patient samples.

REVIEW QUESTIONS: SECTION 4

Answers are provided at the end of this Learning Guide.

1. Which of the following sets of values for repeat analyses of a QC sample (target value of 50) reflects the best precision?

   A. 50, 51, 52

   B. 50, 52, 56

   C. 48, 50, 52

   D. 44, 50, 53

2. Which of the following sets of values for repeat analyses of a sample (target value of 100) shows the least bias?

   A. 100, 105, 110

   B. 95, 100, 105

   C. 90, 95, 100

   D. 90, 100, 105

3. Method A and Method B for cholesterol both give a value of 200 mg/dL for a serum sample; however, the same QC material analyzed by Method A gives 185 mg/dL and by Method B gives 212 mg/dL. What might cause this?

   A. Method B is biased

   B. Method A is imprecise

   C. Both methods are showing a matrix effect for the QC material

   D. Any of the above answers may be correct

4. What does method traceability mean?

   A. The calibration of a method is linear

   B. The method meets the required error budget

   C. The method's accuracy is linked to a certified method and/or material

   D. The method does not show matrix effects

5. Which of the following analytes has the biggest allowable error budget based on CLIA accuracy ranges?

   A. Albumin

   B. Triglycerides

   C. Chloride

   D. Cholesterol

SECTION 5

SOURCES OF ERROR

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LEARNING OBJECTIVES

After completing this section, you will be able to:

1. Identify examples of preanalytical, analytical and postanalytical errors.
2. Describe common sources of analytical error.
3. Identify strategies to detect interferences and avoid reporting erroneous results.

OVERVIEW

This section summarizes common types of errors encountered in the clinical laboratory testing process and presents some of the strategies used to minimize analytic testing errors.

KEY CONCEPTS

1. Patient preparation and proper collection and handling of specimens are important preanalytical steps to ensure the validity of a test result.
2. Hemolysis, icterus and lipemia (HIL) are three of the most common sources of interfering substances found in blood serum and plasma samples.
   - Hemolysis refers to color of hemoglobin released from destroyed red blood cells.
   - Icterus refers to color from bilirubin.
   - Lipemia refers to turbidity from high lipid concentrations, usually triglycerides.

   If unrecognized, their presence may cause overestimation or underestimation of the analyte concentration.
3. Automated instrumentation includes numerous algorithms to detect potential sources of error and alert the operator.

The reader should consult Appendix B: References for more detailed information about these topics, especially *Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics*, 7th edition, 2015.

THREE DIVISIONS FOR SOURCE OF ERROR

Sources of error are typically divided into three categories: preanalytical, analytical and postanalytical. Preanalytical errors are those that occur during sample collection, transport or processing prior to the analysis step. Analytical errors are those that occur during analysis. Postanalytical errors are those that occur after the analysis has taken place.

Preanalytical	
Test orders	Wrong test ordered Test order misunderstood
Patient	Patient improperly prepared Patient misidentified
Collection	Specimen collected in wrong container or collection tube additive Specimen mislabeled Inadequate amount of specimen collected
Transport	Specimen transport under improper conditions Delay in processing and transport time too long Inadequate centrifugation time
Analytical	
Testing	Instrument not calibrated correctly Interfering substances present and not recognized Dilution error Bubbles or particles present in sample
Postanalytical	
Reporting	Result reported incorrectly or with inappropriate units of measure Result sent to wrong location Report delayed or incomplete

PREANALYTICAL ERRORS THAT CAN AFFECT THE ACCURACY OF A TEST RESULT

PATIENT PREPARATION

Many tests require special patient preparation like fasting or avoidance of certain foods, dietary supplements or drugs. It is important that patients follow these instructions so that the test result can be compared in a meaningful way to the reference interval.

For example, the reference interval for triglycerides is based on a specimen collected after 8–12 hours of fasting (no food or drink other than water). If a patient eats a meal or snack shortly before the blood sample is taken, the triglycerides may be higher than the reference range, erroneously suggesting that the patient demonstrates dislipidemia (abnormal concentration of a lipid fraction).

SPECIMEN COLLECTION

Improper specimen collection can affect the test result. Prolonged tourniquet application time can lead to unrepresentative amounts of certain substances in the specimen. These are mostly high molecular weight substances such as proteins, lipids and protein-bound substances like calcium. Use of the wrong anticoagulant for a blood sample, or the wrong preservative for a urine sample, may lead to inaccuracies, either due to a failure to stabilize the analyte or by direct interference in the testing step. Assay package inserts often specify the acceptable types of collection tubes (e.g., glass or plastic, with or without gel separator, preferred tube additive or preservative). A patient specimen collected using a different type of tube may produce inaccurate results.

For example, if 24-hour urine samples for calcium or magnesium testing are not adequately acidified by the addition of hydrochloric acid or other acceptable preservatives, insoluble salts of these metal ions may form and precipitate out of solution. Since the test only measures the ions remaining in solution, the result will underestimate the actual amount.

TRANSPORT

Analyte concentrations may change during the time between collection of the specimen and delivery to the laboratory for analysis. Some samples can be stabilized by refrigeration, some may require freezing, others may need protection from light, and still others might require analysis within limited time frames. If handled incorrectly, the analyte concentration may change and the analyzed sample will not accurately reflect the patient's status.

Ammonia can form from protein breakdown, especially in blood cells. If a blood sample is not immediately placed on ice, the continued formation of ammonia during transport may cause falsely high results suggesting liver disease when no disease is present. Glucose, on the other hand, is consumed by blood cells when a sample of whole blood is stored at room temperature. Significant amounts of glucose can disappear in a matter of 30–60 minutes, risking a failure to recognize high glucose or to falsely identify someone as having low glucose.

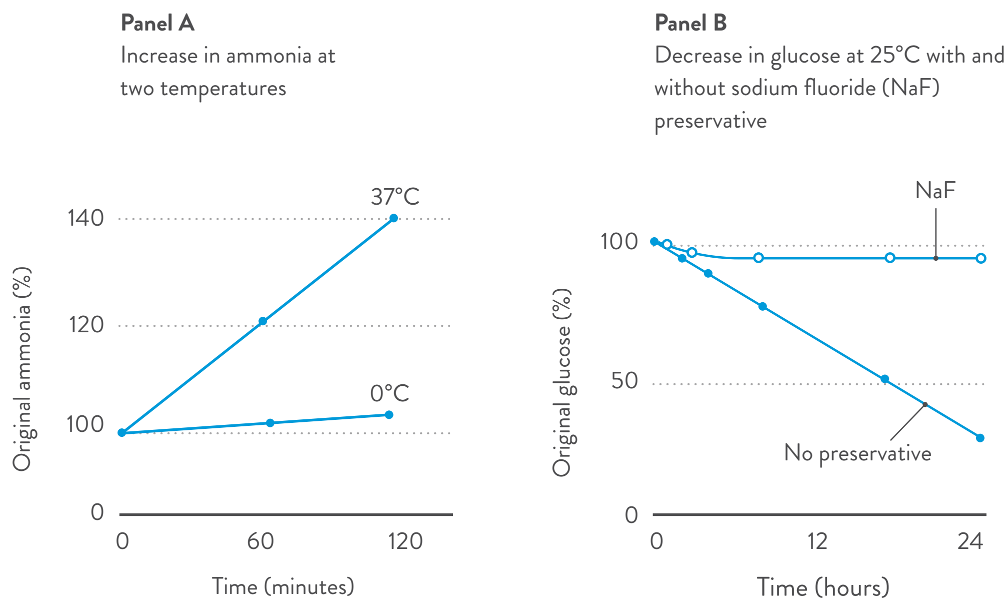


Figure 5-1: Specimen collection — changes in analyte concentration.

ANALYTICAL ERRORS THAT CAN AFFECT THE ACCURACY OF A TEST RESULT

METHOD IS "OUT OF CONTROL"

Methods must be accurately calibrated and their performance verified by a quality control program (Section 4). Sometimes it is not discovered that an assay is out of control until after patient samples have been analyzed. If the method fails to meet performance standards ("out of control"), the results from testing patient samples will be erroneous. In this case, the cause of the problem must be identified and corrected and then the patient samples retested.

INTERFERING SUBSTANCES

Specimens may contain substances that interfere with testing, either by directly absorbing or diffracting light or by reacting with reagents and preventing those reagents from reacting with the intended analyte. Part of the evaluation of test method accuracy includes evaluation of the effect of potential interferents.

HIL

The three most common interferents in a serum or plasma sample:

- Hemoglobin that has leaked from red blood cells and produces a reddish color
- Icteric (or bilirubin): a yellow byproduct of hemoglobin breakdown and metabolism
- Lipemia: extremely high triglycerides that make the sample turbid (cloudy)

Samples that contain these substances are referred to as hemolyzed, due to the breakdown of red blood cells, icteric (another term for high bilirubin), and lipemic, due to the presence of high lipid concentrations. The indices representing these conditions are abbreviated by the letters H, I and L.

Visual inspection of the serum or plasma sample can identify these conditions. Normal serum or plasma is a light straw-colored clear fluid. Hemolysis results in the sample appearing red; icterus as yellow to brown; and lipemia results in a milky or turbid appearance. Qualitative visual scales, ranging from 1+ to 4+, indicate the relative degree of each of these conditions.

The color or turbidity of the interferent can alter the readings taken by a spectrophotometer so the absorbance signal does not reflect the true concentration of analyte. This can lead to a falsely high or falsely low test result.

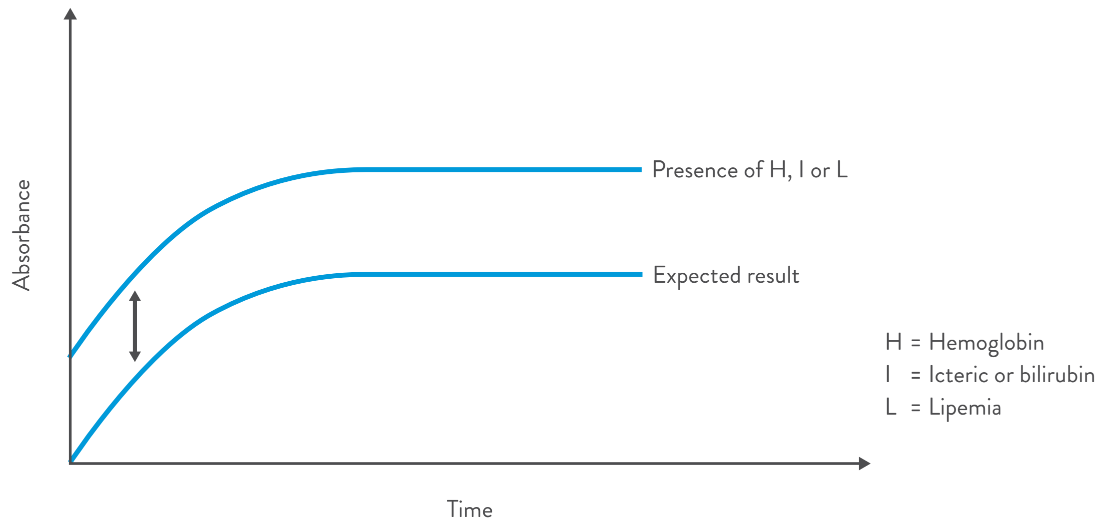


Figure 5-2: Effect of the presence of H, I or L.

The presence of hemoglobin that has leaked from damaged blood cells can also signal the presence of other analytes that have similarly leaked from inside cells to the serum or plasma. These may include potassium, iron, magnesium and intracellular enzymes like lactate dehydrogenase (LD) or aspartate aminotransferase (AST).

Since each of the HIL interferents absorbs light, the amount of these interferents can be estimated using photometric measurements of absorbance at several different wavelengths. A mathematical algorithm can then be used to compute the relative amount of each interferent and provide a semiquantitative estimate. This estimate can be made during the background reading time, before any active reagents are added, or as a separate test.

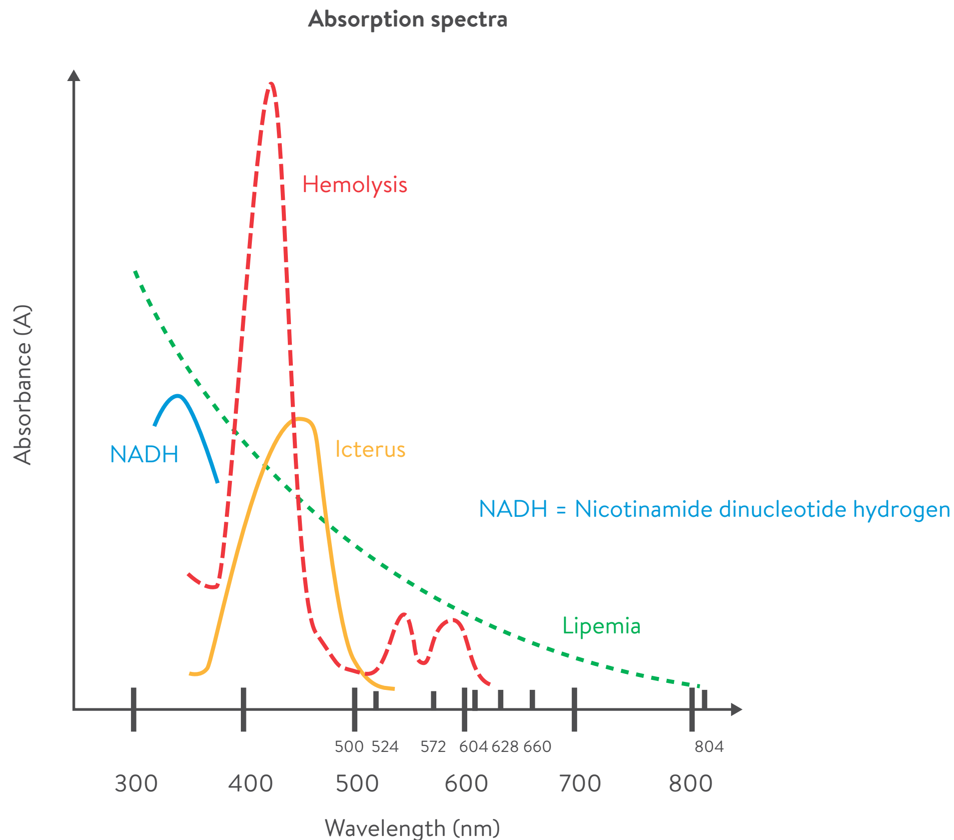


Figure 5-3: Absorbances due to hemoglobin, icteric (bilirubin) and lipemia may be measured in regions utilized for spectral interpretation of analyte, and thus their absorbance can be mistaken for the dye being measured. By taking absorbance measurements at the seven designated wavelengths, the concentrations of each of these interferents can be estimated.

The level of H, I or L present can be linked with information about a test method's susceptibility to the interferent that was collected during evaluation of test accuracy. For example, if the test for potassium has been found to be elevated when H > 2+, but not when L or I are elevated, the laboratory may decide not to report potassium results if H > 2+, or it may choose to report the result with a conditional statement indicating the result is likely to be overestimated because of the high H index.

HIL INDICES

Some analyzers are capable of providing a semiquantitative estimate of HIL concentration, as opposed to the qualitative 1+ to 4+ scale, using differential spectrophotometry. Assay package inserts may define the HIL index units and describe how they approximate the HIL concentrations. The table below is an example of how the qualitative and semiquantitative HIL indices might compare. Note that although the HIL indices may be reported with quantitative units (e.g., mg/dL or g/L), these are only approximate estimates of concentration. A physician can interpret the test result in light of the potential effect of HIL or can request that a new specimen, free of interferents, be collected and tested. If two or more of the HIL interferents are present, the estimates will likely be unreliable and it is advisable to request that a new specimen be collected.

Scale	H index (mg/dL)	I index (mg/dL)	L index (mg/dL)
Blank	< 30	< 2	50
1+	30 ≤ H < 100	2 ≤ I < 4	50 ≤ L < 100
2+	100 ≤ H < 200	4 ≤ I < 10	100 ≤ L < 150
3+	200 ≤ H < 500	10 ≤ I < 20	150 ≤ L < 200
4+	≥ 500	≥ 20	≥ 200

HETEROPHILE ANTIBODIES

Occasionally, patients will have antibodies in their blood that inadvertently cross-react with antibodies in an immunoassay. The presence of these antibodies (called heterophile antibodies) can lead to a falsely high or falsely low result. A common example is HAMA (human anti-mouse antibodies), which are anti-murine antibodies that develop spontaneously in some patients if exposed to mouse antigens. Patients may develop heterophile antibodies if they receive immunotherapies, a vaccine containing serum from another species, or even through environmental exposure. Sometimes the only clue that such an antibody is present is the inconsistency of the test result with the patient's condition. If a provider questions the result, it is possible to repeat the test, adding an anti-heterophile antibody or a blocking substance that will bind to the heterophile antibody in the sample before analysis. The anti-heterophile antibody or blocking substance binds to the heterophile antibody in the patient sample and prevents it from interfering in the test. Some immunoassays include anti-heterophile antibodies or blocking agents in the reagents for the test, to reduce the possibility of interferences from heterophile antibodies in the patient sample.

ANALYTE CONCENTRATION IS HIGHER THAN THE MEASUREMENT RANGE FOR THE TEST

When the analyte concentration is higher than the measurement range (also known as the dynamic range or analytical measurement range [AMR]) of the test, the laboratory has two options. One is to report the value as higher than the upper limit of the test method. This approach is acceptable when only knowing that a result is elevated is sufficient for medical management. However, sometimes it is important to know the exact quantitative value. In such cases, the usual approach is to dilute the sample and reanalyze a diluted aliquot of the sample, mathematically correcting the measured result by a dilution factor.

For example, if the original sample is diluted by taking 1 mL of sample and adding 9 mL of an appropriate diluent (a term for the solution used for making dilutions), the measured result in the diluted sample will be multiplied by 10 to give the value for the original sample.

Manual dilution and reanalysis steps are often undesirable because they are subject to human error such as mismeasurement, miscalculation and use of the wrong diluent. Some tests are sensitive to the diluent so the proper diluent and water, saline or even the zero calibrator may be required for specific tests. Manual dilution and reanalysis can also introduce inefficiencies such as delayed reporting of results and delays to other sample testing. Automated chemistry analyzers often include automatic dilution for determining the concentrations of out-of-range samples without human intervention.

AUTOMATED DILUTION (AUTODILUTION)

When an instrument recognizes that a result is out of range, it may be programmed to prepare a dilution of the out-of-range sample and analyze the diluted sample. If the diluted sample gives a result that is in range, the instrument performs a calculation to correct the reported result with the dilution factor. While this process takes additional time for the dilution and reanalysis, it offers the advantage of minimizing errors and promptly addressing the issue and providing a quantitative result for the sample within minutes. No manual intervention is needed.

RATE ALGORITHMS

For some enzyme rate reactions whose absorbance signal is monitored over the duration of the reaction time, an alternate approach may be taken. Two different read windows, the routine reaction read window and an earlier read window, may be monitored to observe the reaction rate. If the sample has a high concentration of analyte, the added enzymatic substrate reagent is quickly consumed, resulting in substrate depletion. The reaction no longer reflects the true enzyme quantity or activity and cannot be measured. The result is reported as greater than linearity (too high to measure) or, in some cases, if substrate depletion is not detected, as a falsely low result.

This illustration explains the use of two read windows — a main, or routine, read window and an earlier read window — to accurately determine the enzyme activity. For example, if less than three linear data points fall within the main read window, the system will use the earlier read window to calculate the result. The earlier read window can use an algorithm to calculate the concentration of enzyme activity while the reaction is still in the linear range.

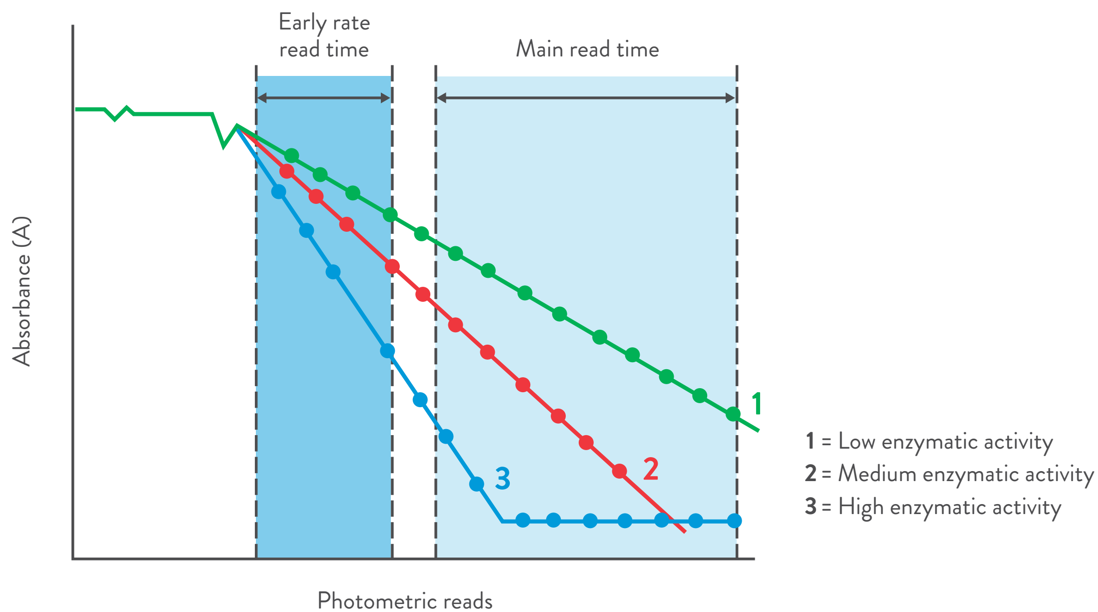


Figure 5-4: Use of alternate read windows to extend linearity of enzymes.

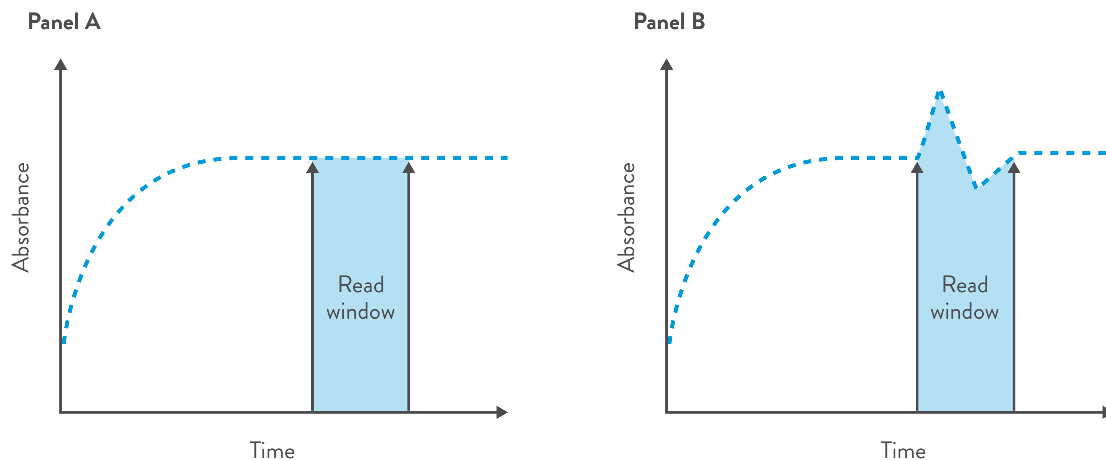
RANDOM ERRORS

Random errors are errors that occur as the result of unpredictable events that affect the measurement of the signal. Examples of random errors include air bubbles or particulate matter in the sample resulting in pipetting a too small volume of sample. Random errors are unpredictable.

- A bubble in the sample may result in too little sample, and the result of the test will underestimate the amount of analyte.
- A bubble in the light path of the spectrophotometer may cause an increase or decrease in absorbance and may lead to underestimation or overestimation of the analyte concentration.
- Particulate matter, often microclots in a plasma sample that was poorly collected or inadequately mixed with anticoagulant, may impede the measurement of an aliquot for testing by clogging the sampling probe or, like bubbles, by displacing some of the liquid.
- Microclots may also diffract light and cause errors in the absorbance readings.
- "Short sampling," using less than the required volume of patient specimen in the reaction mixture, causes falsely low results.

Fortunately, many automated analyzers are programmed to recognize the presence of bubbles, microclots, low sample volume or other random errors. For example, the sampling probe that measures the aliquot for analysis can sense when the sample is not flowing at the expected rate, as it might be if impaired by the presence of a microclot, and generate a pressure monitoring error for the analysis.

Spectrophotometric readings are monitored with time. Instruments can recognize if the absorbance signal is not demonstrating the expected steady increase or decrease during the reaction time and is instead showing some random high or low values, as would be seen with a bubble or particle floating through the light path. When bubbles, clots or other random events lead to unexpected sampling or signal patterns, the instrument can alert the operator that this test result is suspect and needs to be retested.



Expected absorbance increases are smooth, regular curves (Panel A). The presence of bubbles, foam or particle in the photometric window will cause sporadic high or low values (Panel B).

Figure 5-5: Random error from bubbles, foam or precipitates.

REVIEW QUESTIONS: SECTION 5

Answers are provided at the end of this Learning Guide.

1. Which of the following is an example of a preanalytical error?
   - A. Test method incorrectly calibrated
   - B. Collection of blood in wrong kind of tube
   - C. Presence of interfering substance in specimen
   - D. Delay in sending the report to the provider

2. Which type of analytical error can be prevented by a good quality control program?
   - A. Instrument not properly calibrated
   - B. Presence of interfering substances in sample
   - C. Presence of bubbles in the light path of a photometric method
   - D. Analyte concentration so high it depletes the active reagent

3. Which type of analytical error is recognized by an HIL index?
   - A. Instrument not properly calibrated
   - B. Presence of interfering substances in sample
   - C. Presence of bubbles in the light path of a photometric method
   - D. Analyte concentration so high it depletes the active reagent

4. What option(s) might be employed if a test result is above the upper limit of the test measurement range?
   - A. Manual dilution followed by reanalysis of the diluted sample
   - B. Automatic dilution and reanalysis of the sample
   - C. Use of a reaction rate algorithm using two read windows for an enzyme assay
   - D. Reporting the result as higher than the upper limit of the test method

SECTION 6

COMMON CLINICAL CHEMISTRY TESTS

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LEARNING OBJECTIVES

After completing this section, you will be able to:

1. Identify what analyte is being measured in common chemistry tests.
2. Explain when certain tests might be ordered.
3. Identify medical conditions that might lead to a high or low test result.

OVERVIEW

This section identifies analytes that most commonly comprise clinical chemistry test menus and describes their metabolic function and some pathological conditions for which the analyte might be measured.

KEY CONCEPTS

1. Clinical chemistry tests measure a wide variety of analytes that reflect many different organ systems and diseases.
2. Some test results are specific indicators for a single organ system or disease; others are general indicators of a disease or disorder, but do not pinpoint the specific organ or disease process.
3. Tests are performed for different reasons. Some tests help diagnose a disease, others monitor the course of the disease progression or effectiveness of therapy, and still others are used to screen for risk of developing a disease.

Hundreds of compounds, molecules and ions circulate in body fluids. Many of these can be measured by tests used in clinical chemistry laboratories. These tests are valuable in the prevention, diagnosis and treatment of disease.

This section gives only a sampling of some of the more common analytes that are measured in the clinical laboratory. They are grouped by the type of analyte being measured. These range from ions to small molecules to proteins (macromolecules) to lipids and lipoproteins that circulate in complexes containing hundreds of molecules and macromolecules.

Reference ranges or expected results for healthy adult individuals are provided as a guide for discussion in this section. These values were sourced from the 7th edition of *Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics* unless otherwise stated. These values may differ with different patient populations, regions of the world and assay methodologies, and should be verified by laboratories prior to use.

ELECTROLYTES AND IONS

Electrolytes and ions are small charged particles; cations are positively charged and anions are negatively charged. They are found in all body fluids — both inside of cells and in extracellular fluids. They maintain osmotic pressure (pressure across membranes or between different fluid compartments) and fluid balance, and play an important role in many metabolic processes.

MEASURING ELECTROLYTES

The tests shown below constitute the group commonly referred to by the terms "electrolyte panel" or "LYTES." Electrolytes help regulate water balance and acid-base balance in the body. These tests are most often ordered together to assess overall electrolyte balance — often in critical care settings as well as in routine settings. Some conditions in which electrolyte balance is of concern include edema, weakness, confusion, cardiac arrhythmias, high blood pressure, heart failure, liver disease and kidney disease. Electrolyte panels often include a calculated value termed "anion gap" that may indicate the presence of unmeasured anions in the blood.

Electrolytes

Analyte	Description	Typical reference intervals for healthy adults*	Reasons for increased and decreased values
Sodium (Na+)	Major extracellular cation responsible for maintaining fluid balance in circulation; blood levels are controlled through excretion and resorption by the kidneys	136–145 mmol/L	↑ Dehydration, Cushing's syndrome, diabetes insipidus ↓ GI loss (diarrhea and vomiting), Addison's disease, renal disease
Potassium (K+)	Major intracellular cation responsible for muscle contraction and maintenance of normal heart rate	3.5–5.1 mmol/L	↑ Shock, circulatory failure, renal disease ↓ GI loss (vomiting and diarrhea), diuretic use, some cancers
Chloride (Cl-)	Major extracellular anion; changes in concentration typically mirror sodium concentrations	98–107 mmol/L	↑ Dehydration ↓ Low blood sodium, vomiting
Carbon dioxide (CO_2)	Major anion that buffers blood to physiologic pH of 7.4	22–28 mEq/L	↑ Metabolic alkalosis ↓ Metabolic acidosis
Anion gap	The anion gap is a calculated value; one formula for it is: Anion gap = sodium — (chloride + bicarbonate); an alternate formula includes potassium with sodium; a high anion gap reflects the presence of unmeasured anions, possibly from an acute or chronic disease process from ingestion of a toxic substance	7–16 mmol/L	↑ Ketoacidosis (starvation, uncontrolled diabetes), lactic acidosis, toxicity from ingestion of substances such as alcohol, salicylates, ethylene glycol (antifreeze), oxalate

*Typical values for healthy adults may vary with different patient populations, setting and assay techniques/methodologies. Each lab must verify the range it uses.

OTHER COMMONLY MEASURED IONS

Several other ions, not part of the electrolyte panel, are common tests in clinical chemistry. Like the electrolytes, these ions are found in many different tissues and serve many different metabolic functions.

Ions

Analyte	Description	Why measured	Typical reference intervals for healthy adults*	Reasons for increased and decreased values
Calcium (Ca^{++})	Mineral required for bone formation and for blood clotting and important in nerve and muscle function	Often measured as a screening test since it is usually tightly controlled and held in a very narrow concentration range; abnormalities can signal a wide range of metabolic problems	8.6–10.3 mg/dL 2.15–2.50 mmol/L	↑ Hyperparathyroidism, some cancers, excess vitamin D intake ↓ Hypoparathyroidism, vitamin D deficiency, chronic kidney disease, pancreatitis
Phosphorus (Phosphate) (PO_4^{-3})	Mineral important in bone metabolism, energy production, and nerve and muscle function	Usually measured along with other analytes to help diagnose problems with calcium metabolism	2.7–4.5 mg/dL 0.87–1.45 mmol/L	↑ Renal insufficiency, vitamin D overdose, high phosphate intake ↓ Overuse of diuretics or antacids, hyperparathyroidism
Magnesium (Mg^{++})	Essential mineral for the function of many enzymes, especially those converting energy for muscle function; also important in bone structure	Often ordered as a follow-up test for low calcium or potassium or to assess symptoms of muscle problems like weakness, twitching, cramping or cardiac arrhythmias	1.6–2.6 mg/dL 0.66–1.07 mmol/L	↑ Renal disease, severe dehydration ↓ Malabsorption, pancreatitis, diarrhea, alcoholism ↑ Eclampsia, treatment and obstetric emergencies
Iron (Fe^{++})	Critical component of oxygen transport proteins like hemoglobin and myoglobin; also a part of many enzymes involved in energy pathways	Used primarily to determine "iron status"; to determine if patient is iron deficient or to determine if patient has an iron overload syndrome	Male 66–175 μg/dL 11.6–31.3 μmol/L Female 50–170 μg/dL 9–30.4 μmol/L	↑ Multiple blood transfusions, iron injections, hereditary hemochromatosis ↓ Iron-poor diet, loss of blood, anemia

*Typical values for healthy adults may vary with different patient populations, setting and assay techniques/methodologies. Each lab must verify the range it uses.

SMALL MOLECULES AND METABOLITES

Formation (anabolism) and breakdown (catabolism) of biologic molecules is central to life. Every living organism uses molecules as sources of energy, as building blocks for cells and tissue, and as metabolic sensors to control metabolism.

Thousands of small molecules (for this section, we will consider small as below a molecular weight of 1,000) are created and destroyed in metabolic processes every day. Those that circulate in blood or that are excreted in urine can be useful indicators of how well the body is functioning — whether the patient is using and storing energy efficiently, is eliminating waste products, and is healthy. Several commonly measured small molecules include those that reflect nutritional status, those that reflect the elimination of waste products and those that reflect metabolic control. Some examples of each type are given in the tables below.

Small molecules: nutrition

Analyte	Description	Why measured	Typical reference intervals for healthy adults*	Reasons for increased and decreased values
Glucose	A major source of energy for many tissues — regulated by hormones, such as insulin, cortisol and glucagon	To test for diabetes (high blood glucose reflecting insulin deficiency or insulin resistance); in critical care setting to test metabolic state	Target fasting value 74–106 mg/dL 4.1–5.9 mmol/L	↑ Diabetes, Cushing's disease, stress ↓ Insulin excess, starvation, adrenal insufficiency
Vitamin B_{12}	Required for red blood cell function and important in nerve function	To identify a deficiency when low iron and large red blood cells are present (macrocytic anemia) and to monitor therapy for low vitamin B_{12}	200–835 pg/mL 148–616 pmol/L	↑ Some leukemias ↓ Malnutrition, malabsorption, pernicious anemia
Folic acid	Needed for red blood cell function and important for cell division, especially needed in pregnancy for the developing fetus; deficiency can lead to neural tube defects	Measured with vitamin B_{12} to determine the cause of macrocytic anemia, and to monitor therapy for low folate	3–20 ng/mL 7–45 mmol/L	↑ Pernicious anemia ↓ Malnutrition, malabsorption (e.g., in celiac disease or alcoholism)

*Typical values for healthy adults may vary with different patient populations, setting and assay techniques/methodologies. Each lab must verify the range it uses.

Small molecules: waste products

Analyte	Description	Why measured	Typical reference intervals for healthy adults*	Reasons for increased and decreased values
Total bilirubin**	This breakdown product of hemoglobin is excreted by the liver into the bile — it circulates in blood in two forms referred to as conjugated and unconjugated, reflecting whether the carboxyl groups are free (unconjugated) or esterified (conjugated)	To assess liver function	0.1–1.2 mg/dL 2–21 μmol/L	↑ Hepatitis, cirrhosis, hemolytic diseases, and obstruction of biliary or hepatic ducts
Direct bilirubin** (Conjugated bilirubin)	The conjugated form is water-soluble, made in the liver and excreted in the blood; it reacts directly with diazo dyes, so it is called direct reacting	To test the liver's ability to conjugate bilirubin and excrete it	< 0.3 mg/dL < 5 μmol/L	↑ Obstruction of biliary or hepatic ducts, and in hereditary conditions like Dubin-Johnson syndrome
Indirect bilirubin** (Unconjugated bilirubin)	The unconjugated form is fat-soluble and the product of hemoglobin breakdown; it reacts with diazo dyes only in the presence of activators, so it is called indirect reacting	Indirect bilirubin is calculated as Total Bilirubin — Direct Bilirubin; it reflects the difference between the total and direct forms	0.1–1.0 mg/dL 2–17 μmol/L	↑ Hereditary conditions like Gilbert's disease and Crigler-Najjar syndrome
Neonatal bilirubin	Unconjugated bilirubin found in newborns whose livers are too immature to conjugate bilirubin; in neonates, unconjugated bilirubin can enter the fatty tissue of the brain and central nervous system and cause brain damage (mental retardation, hearing defects or cerebral palsy)	Newborns are routinely tested for bilirubin to determine whether intervention is necessary to lower bilirubin and thereby lower the risk of brain and nervous system damage	3–5 days old, premature: < 16 mg/dL < 274 μmol/L 3–5 days old, full term: 1.5–12 mg/dL 26–205 μmol/L	↑ Hemolysis of red blood cells such as with Rh incompatibilities, kernicterus

*Typical values for healthy adults may vary with different patient populations, setting and assay techniques/methodologies. Each lab must verify the range it uses.

**Reference intervals sourced from *Clinical Diagnosis and Management by Laboratory Methods*, 19th edition.

Small molecules: waste products

Analyte	Description	Why measured	Typical reference intervals for healthy adults*	Reasons for increased and decreased values
Lactic acid	This metabolite is released from muscle under conditions of anaerobic energy production; if high amounts are released into the blood, it can disrupt acid/base balance (lactic acidosis)	To evaluate symptoms that suggest poor oxygen delivery to tissue such as shortness of breath and muscle weakness; to evaluate patients in shock, congestive heart failure or coma	< 18 mg/dL** < 2.0 mmol/L**	↑ Shock, muscle fatigue, diabetic ketoacidosis, tissue hypoxia, severe infections like meningitis
Uric acid	A waste product from breakdown of purines (DNA components) that is excreted by the kidneys; too much uric acid can lead to deposits of urate crystals in joints (gout) or in kidneys (kidney stones)	To evaluate joint inflammation that may be due to gout; often ordered to monitor uric acid production in patients undergoing chemotherapy or radiation treatments	Male 3.5–7.2 mg/dL 0.21–0.42 mmol/L Female 2.6–6.0 mg/dL 0.15–0.35 mmol/L	↑ Gout, kidney disease, leukemia
Creatinine	A waste product from the muscle breakdown of a compound called creatine, which is excreted into urine by the kidneys	To evaluate kidney function and monitor therapy for kidney disease; creatinine may be measured in blood, urine or both to evaluate kidney function	Male 0.7–1.3 mg/dL 62–115 μmol/L Female 0.6–1.1 mg/dL 53–97 μmol/L	↑ Kidney dysfunction that may be due to a variety of different causes, such as drug toxicity, poorly controlled diabetes or inadequate blood flow through the kidneys, as seen with shock or congestive heart failure
Urea nitrogen (BUN)	A waste product from protein breakdown formed by the liver and excreted by the kidney	Often ordered with creatinine to evaluate kidney function; also used to monitor dialysis patients	6–20 mg/dL 2.1–7.1 mmol/L	↑ Kidney dysfunction, stress, high protein diets ↓ Low protein diets, liver disease
Ammonia*** (NH4+)	A waste product of amino acid breakdown converted to urea by the liver; increased ammonia levels can cause mental and neurologic changes in the brain	To evaluate disorientation, confusion and coma in adults; to evaluate irritability, lethargy and seizures in newborns	40–80 μg/dL 23–47 μmol/L	↑ Severe liver disease, cirrhosis, severe hepatitis, Reye's syndrome, inherited genetic deficiencies

*Typical values for healthy adults may vary with different patient populations, setting and assay techniques/methodologies. Each lab must verify the range it uses.

**Lactic acid is from: AJ, R., SW, L., SR, B., & JA, G. (2015, September). *Lactic acidosis: Clinical implications and management strategies. Cleveland Clinic Journal of Medicine*, 82(9), 615-624.

***Reference intervals sourced from *Clinical Diagnosis and Management by Laboratory Methods*, 20th edition.

Small molecules: hormones

Analyte	Description	Why measured	Expected values*
Thyroid stimulating hormone (TSH)	Made by the pituitary gland, it maintains stable amounts of thyroid hormones (T3 and T4) in the blood	To screen for or diagnose thyroid disorders; to monitor treatment of thyroid disease	Expected values fall between 0.4 and 4.2 mIU/mL; if TSH is used for monitoring treatment, a high-sensitivity or third-generation test called hs-TSH is better able to measure TSH at very low levels; ↓ TSH is hyperthyroid with symptoms of anxiety, weight loss, tremors, weakness, light sensitivity, puffiness around eye/bulging eyes; ↑ THS is hypothyroid with symptoms of weight gain, dry skin, cold intolerance, hair loss and fatigue
Thyroxine (T4) and triiodothyronine (T3)	T4 is made by the thyroid gland and converted to T3 by the liver; these hormones control energy production (metabolic rate) in tissues; both circulate with the majority bound to protein	As follow-up to an abnormal TSH test; lab tests may measure either the total hormone or free hormone (not bound to protein)	Interpretation of T4 and T3 depend on the pattern of results from all three tests — TSH, T4 and T3 (see Section 7 for more detail)
Estrogen	A female hormone, produced by the ovaries, that triggers ovulation	To evaluate causes of infertility and to assess menopause	Reference values differ with the menstrual cycle phase; for females, ↓ levels are seen after menopause, dysfunction of ovaries, failing pregnancy and polycystic ovarian syndrome; ↑ early puberty and tumors of ovaries and adrenal glands; for males, ↑ tumors of testicles or adrenal glands, delayed puberty and enlarged breasts; both sexes, ↑ hyperthyroidism and cirrhosis; ↓ Turner syndrome
Testosterone	Hormone responsible for development of male secondary sex characteristics; in females, it is converted to estradiol, the major female sex hormone	To evaluate infertility or erectile dysfunction in adult males; in boys, to investigate delayed puberty; in females, to investigate development of male traits (virilization)	Before age 10, values are similar in males and females; values increase with age, especially in males, where there is a dramatic rise at puberty; adult males have much higher testosterone levels than adult females; test results must be compared to an appropriate reference value based on sex and age; ↓ hypothalamic or pituitary disease, some genetic diseases associated with testicular failure and infertility or chronic disease; males ↑ testicular or adrenal tumors, early puberty, congenital adrenal hyperplasia in babies and children, use of anabolic steroids; females ↑ polycystic ovarian syndrome, ovarian or adrenal tumors, and congenital adrenal hyperplasia
Beta-human chorionic gonadatropin (β-HCG)	β-HCG is a protein produced in the placenta during pregnancy; in a non-pregnant woman, no β-HCG is detectable; in pregnancy, β-HCG doubles every 2 to 3 days during the first weeks of pregnancy	A test for β-HCG in either blood or urine is used to confirm pregnancy	Tests detecting β-HCG may be qualitative or quantitative — qualitative tests can usually detect pregnancy about 10 days after a missed period; quantitative tests are most often used to detect an ectopic pregnancy or to monitor a woman after miscarriage; ↑ pregnancy, ectopic pregnancy, gestational trophoblastic disease and germ cell tumors

*Typical values for healthy adults may vary with different patient populations, setting and assay techniques/methodologies. Each lab must verify the range it uses.

PROTEINS

Proteins are macromolecules — polymers that are formed from essential amino acids. Most proteins are large — with molecular weights ranging from 30,000 to more than 500,000. They are integral parts of every cell, fluid and organ in the body.

Proteins that are the focus of clinical chemistry analyses are primarily those that circulate in the blood. These include plasma proteins, transport proteins, defense proteins and clotting proteins, which function primarily in the circulation and extracellular fluid. Most of these proteins are made by the liver, with the exception of immunoglobulins, which are made by immune cells (specifically B lymphocytes).

Other proteins sometimes found in blood are proteins whose primary functions are intracellular. They may have leaked from the inside of the cells where they were made and their presence in blood often reflects some kind of damage to the cell.

Proteins: general and transport

Analyte	Description	Why measured	Typical reference intervals for healthy adults*	Reasons for increased and decreased values
Total protein, serum/plasma	Measures the amount of proteins, primarily albumin and globulins, found in serum or plasma, maintains circulatory system oncotic pressure	It is a screening test to see if protein levels are at expected value	6.4–8.3 g/dL 64–83 g/L	↑ Dehydration, infections, some cancers like myelomas and lymphomas ↓ Protein loss (from kidney or GI tract), liver disease, malnutrition
Urine protein	Normally very little protein is found in urine; if protein level is high, the kidneys are failing to retain/reabsorb protein appropriately	To evaluate kidney function; it is often used to monitor patients taking certain nephrotoxic drugs	50–80 mg/day	↑ Kidney failure (nephrotic syndrome), diabetes
CSF protein**	Protein in cerebrospinal fluid; components are similar to those in blood plasma	Used to investigate possible diseases of the central nervous system	12–60 mg/dL 120–600 mg/L	↑ Meningitis, tumors of brain or spinal cord, multiple sclerosis, Guillain-Barré syndrome

*Typical values for healthy adults may vary with different patient populations, setting and assay techniques/methodologies. Each lab must verify the range it uses.

**Reference intervals sourced from *Clinical Diagnosis and Management by Laboratory Methods*, 20th edition.

Proteins: general and transport

Analyte	Description	Why measured	Typical reference intervals for healthy adults*	Reasons for increased and decreased values
Albumin, serum/plasma	Major protein in blood made in the liver; it binds and transports many substances	Albumin level is a general indicator of health and nutritional status	3.5–5.2 g/dL 35–52 g/L	↑ Dehydration, infection, malignancy ↓ Starvation, burns, kidney disease, liver disease
Albumin urine** (microalbumin)	Albumin is too large to escape from plasma into urine; its presence in urine signals a problem with the glomerular filtration system of the kidney	Used to monitor kidney function and to screen for early stages of kidney dysfunction in diabetics or people with high blood pressure	15–150 mg/24 hours	↑ Kidney disease
Globulins**	Term for proteins in the blood other than albumin	Calculated as Total Protein – Albumin, globulins are the other major protein fraction	2.3–3.5 g/dL 23–35 g/L	↑ Infections, multiple myeloma ↓ Leukemias, immunosuppression
Prealbumin (transthyretin)	Protein that is unrelated to albumin; it binds thyroxine — it is involved in vitamin A transport and rises and falls rapidly with nutritional status	Nutritional marker often used to assess nutritional status of hospitalized patients or patients scheduled for surgery; it is not affected by hydration status	10–40 mg/dL 100–400 mg/L	↓ Malnourishment, liver disease, inflammation
Ferritin	Iron storage protein found primarily inside cells	Ferritin is often tested along with iron and transferrin to assess iron status	Male 20–250 μg/L or ng/mL Female 10–120 μg/L or ng/mL	↑ Iron overload, inflammation, multiple blood transfusions ↓ Iron deficiency

*Typical values for healthy adults may vary with different patient populations, setting and assay techniques/methodologies. Each lab must verify the range it uses.

**Reference intervals sourced from *Clinical Diagnosis and Management by Laboratory Methods*, 20th edition.

Proteins: general and transport

Analyte	Description	Why measured	Typical reference intervals for healthy adults*	Reasons for increased and decreased values
Transferrin	Primary protein for transport of iron; made by the liver	Test for iron status	215–380 mg/dL 2.15–3.80 g/L	↑ Iron deficiency, pregnancy and oral contraceptives ↓ Hemolytic anemia, malnutrition, inflammation and liver disease
Total iron-binding capacity (TIBC)	Maximum amount of iron that can be transported by the transferrin in blood	Tests iron status; describes amount of transferrin in terms of its iron transport capacity	250–425 µg/dL 44.8–71.6 µmol/L	↑ Iron deficiency, pregnancy and oral contraceptives ↓ Hemolytic anemia, malnutrition, inflammation and liver disease
Unsaturated iron-binding capacity (UIBC)	Reserve capacity of transferrin for additional iron transport	Sometimes used to monitor treatment for iron toxicity	110–370 µg/dL 19.7–66.2 µmol/L	↑ Iron deficiency, pregnancy and oral contraceptives ↓ Hemolytic anemia, malnutrition, inflammation and liver disease
Haptoglobin	Binds and transports free hemoglobin that is released from destroyed red blood cells	Used to distinguish hemolytic anemia (rapid destruction of red blood cells) from other kinds of anemias	26–185 mg/dL 260–1,850 mg/L	↑ Infection or inflammation ↓ Hemolytic anemia, intravascular hemolysis
Ceruloplasmin	Protein that binds and transports copper	Measured along with serum and/or urine copper concentrations to test for diseases of copper metabolism, like Wilson's disease	18–45 mg/dL 180–450 mg/L	↑ Inflammation, pregnancy ↓ Wilson's disease, copper deficiency

*Typical values for healthy adults may vary with different patient populations, setting and assay techniques/methodologies. Each lab must verify the range it uses.

IMMUNOGLOBULINS

Immunoglobulins are circulating antibodies essential for defense against foreign substances. They recognize specific antigenic structures on proteins, viruses or bacteria; bind to these; and initiate a series of reactions (termed immune response) designed to disable and destroy the antigen. Immunoglobulins are termed monoclonal and polyclonal. Monoclonal immunoglobulins are produced by a single line of white blood cells (B-cells), and all have exactly the same chemical composition, sequence and structure. Polyclonal refers to the aggregate collection of monoclonal immunoglobulins produced by many different B-cell lines. Increased levels of polyclonal immunoglobulins are found in infections and inflammations, reflecting a widespread immune response to the infecting agent. Increased monoclonal proteins are seen in malignancies like multiple myeloma, Waldenström's macroglobulinemia and some lymphomas. In these conditions, a single clone of B-cells has become malignant and produces excessive amounts of a single version of an immunoglobulin molecule. Monoclonal immunoglobulins may be IgA, IgG, IgM or IgE.

Proteins: immunoglobulins

Analyte	Description	Typical reference intervals for healthy adults*
IgA**	Protects mucous membranes, found in saliva, tears and sweat — about 10%–15% of serum immunoglobulins are IgA	113–563 mg/dL 1.1–5.6 g/L
IgG**	IgG makes up about 75%–80% of total serum immunoglobulins — IgG confers long-term immunity; it crosses the placenta to give passive protection to the fetus	800–1,801 mg/dL 8–18 g/L
IgM**	IgM is the largest immunoglobulin in size and is the first to form in response to an infection; it comprises about 10%–15% of the circulating immunoglobulins, and activates complement factors to destroy invaders	54–222 mg/dL 0.5–2.2 g/L
IgE	IgE is responsible for allergic reactions by stimulation of histamine production	3–423 IU/L

*Typical values for healthy adults may vary with different patient populations, setting and assay techniques/methodologies. Each lab must verify the range it uses.

**Reference intervals sourced from *Clinical Diagnosis and Management by Laboratory Methods*, 20th edition.

COMPLEMENT

The complement system is a set of circulating blood proteins that function together to promote immune responses that attack and destroy foreign substances like bacteria. The two components most frequently measured are C3 and C4. Complement tests are usually ordered to determine the possible cause of frequent infections or high levels of autoimmune activity.

Proteins: complement

Analyte	Description	Typical reference intervals for healthy adults*
Complement C3	Central protein to all complement activation pathways	83–177 mg/dL 0.83–1.77 g/L
Complement C4	Protein that functions with antigen antibody complexes to activate the complement system	29–68 mg/dL 0.29–0.68 g/L

*Typical values for healthy adults may vary with different patient populations, setting and assay techniques/methodologies. Each lab must verify the range it uses.

CLOTTING PROTEINS

Most clotting proteins are measured in functional assays that detect how well plasma supports clot formations when triggered by a specific agent. These tests, which do not measure specific molecules involved in clotting, are usually not performed in the clinical chemistry section of the lab, but rather the coagulation laboratory. However, there are a few tests that measure specific proteins in the clotting cascade.

Proteins: clotting proteins

Analyte	Description	Why measured
Fibrinogen	Fibrinogen is a soluble protein that is converted by clotting factors into insoluble fibrin threads that stabilize a clot at a site of injury, protecting the site until it heals	To determine if enough fibrinogen is present for normal blood clotting and if fibrinogen has been consumed in a process called disseminated intravascular coagulation (DIC)
D-dimer	D-dimer is a breakdown product of blood clots called fibrin degradation products; specifically, D-dimer consists of cross-linked fibrin fragments	To determine if blood clots have formed in the circulation, especially in DVT (deep vein thrombosis), DIC (disseminated intravascular coagulopathy), and PE (pulmonary embolism), D-dimer is normally not detected in blood; the presence of D-dimer signals inappropriately high levels of clotting

ENZYMES

Metabolic reactions in the body are regulated by biological catalysts called enzymes. Enzymes function primarily in cells. Their presence in blood is usually the result of enzymes leaking from damaged cells.

Proteins: enzymes

Analyte	Description	Why measured	Typical reference intervals for healthy adults*	Reasons for increased and decreased values
Alanine aminotransferase (ALT)	Primarily found in liver	To assess liver disease, more specific for liver diseases than AST	Male 13–40 U/L 0.22–0.68 µkat/L Female 10–28 U/L 0.17–0.48 µkat/L	↑ Hepatitis, cirrhosis, Reye's syndrome, hepatomas (liver cancers), monitor drug-induced liver damage
Aspartate aminotransferase (AST)	Widely present in tissue, especially liver, heart and skeletal muscle	To assess liver disorders	8–20 U/L 0.14–0.34 µkat/L	↑ Liver disease, heart attack, trauma
Alkaline phosphatase** (ALP)	Found in many tissues, especially bone, intestine, kidney and liver	Assessment of bone diseases and liver diseases	20–130 U/L 0.67–2.51 µkat/L	↑ Liver disease, bone disease and periods of bone growth ↓ Low phosphate, hypothyroidism, pernicious anemia
Gamma glutamyl-transferase (GGT)	Present in liver and some other tissue — very sensitive indicator of any liver disorder	Assess liver disease or damage	Male 2–30 U/L 0.03–0.51 µkat/L Female 1–24 U/L 0.02–0.41 µkat/L	↑ Biliary obstruction, alcoholic liver disease
Lactate dehydrogenase (LD)	Widely distributed in tissues like heart, lung, liver, kidney, skeletal muscle; occurs in five forms, numbered LD-1 to LD-5, with different forms predominating in different tissue	General indicator of tissue damage	100–190 U/L 1.7–3.2 µkat/L	↑ Heart attack, liver disease, lung disease, trauma

*Typical values for healthy adults may vary with different patient populations, setting and assay techniques/methodologies. Each lab must verify the range it uses.

**Reference intervals sourced from *Clinical Diagnosis and Management by Laboratory Methods*, 19th edition.

Proteins: enzymes

Analyte	Description	Why measured	Typical reference intervals for healthy adults*	Reasons for increased and decreased values
Creatine kinase (CK)	Muscle enzyme different forms of the enzyme are specific for different kinds of tissue; CK-BB is found primarily in brain and neurologic tissue; CK-MB, primarily in heart muscle; CK-MM, primarily in skeletal muscle	Indicator of muscle damage; the CK-MB enzyme is elevated about 4–6 hours after a heart attack (myocardial infarction [MI]); prior to the availability of the troponin test, CK-MB was used for diagnosis of MI	Male 25–130 U/L 0.43–2.21 µkat/L Female 10–115 U/L 0.17–1.96 µkat/L	↑ Muscle damage, extreme exercise, trauma ↓ People with very low muscle mass
Amylase	Digestive enzyme secreted by salivary and pancreatic glands responsible for digestion of starches	To diagnose pancreatitis	27–131 U/L 0.46–2.23 µkat/L	↑ Acute pancreatitis, blocked pancreatic ducts ↓ Some liver diseases
Lipase	Digestive enzyme secreted by pancreas and salivary glands responsible for breakdown of triglycerides	To diagnose pancreatitis	31–186 U/L 0.5–3.2 µkat/L	↑ Acute or chronic pancreatitis or other pancreatic disease, sometimes with gallstones
Pseudocholine-sterase or cholinesterase activity	Cholinesterases are enzymes that react with succinylcholine, a muscle relaxant used in surgery; people with a genetic deficiency of this enzyme have prolonged, sometimes fatal, reactions to the drug succinylcholine	If genetic deficiency is suspected; if insecticide poisoning is suspected	4.9–11.9 U/mL or kU/L	↑ Insecticide poisoning ↓ Genetic deficiency

*Typical values for healthy adults may vary with different patient populations, setting and assay techniques/methodologies. Each lab must verify the range it uses.

TUMOR MARKERS

Tumor markers are proteins that are selectively produced by and released from cancer (tumor) cells, but not typically from normal cells. The presence of these proteins can be used for screening, helping with diagnosis, staging of disease, monitoring effectiveness of therapy and providing evidence of recurrence. Not all tumor markers can be used for all of these purposes. Relatively few tumor markers are useful for screening asymptomatic populations. Most are used primarily for monitoring treatment and watching for evidence of recurrence. Some common markers are shown in this table. Tumor markers are typically measured using immunoassays, and reference intervals are method specific.

Proteins: tumor markers

Analyte	Types of cancers where the analyte is present	Why measured
Prostate-specific antigen (PSA)	Prostate	• Screen asymptomatic patients • Confirm diagnosis • Monitor therapy • Determine recurrence
Carcinoembryonic antigen (CEA)	Colorectal, lung, breast, liver, pancreas, bladder	• Monitor treatment • Determine recurrence
Cancer antigen 125 (CA 125)	Ovarian	• Confirm diagnosis • Monitor treatment • Determine recurrence
Cancer antigen 15-3 (CA 15-3)	Breast, some ovarian	• Stage the disease • Monitor treatment • Determine recurrence
Alpha-fetoprotein (AFP)	Liver, ovarian, testicular	• Monitor treatment • Determine recurrence

Proteins: special

Analyte	Description	Why measured	Expected values*
Glycated hemoglobin (HbA1c)**	Hemoglobin molecule with a glucose molecule covalently bound	In diabetics, gives a good estimate of glucose control over a 3-month period (the lifetime of a red blood cell)	Normal < 5.7% (< 39 mmol/mol)*** Prediabetes 5.7%–6.4% (39–46 mmol/mol)*** Diabetes ≥ 6.5% (≥ 48 mmol/mol)*** Diabetic good control < 7% (< 53 mmol/mol)***
Troponin I and troponin T	Troponins are intracellular proteins found specifically in heart muscle; they are released when there is damage to cardiac cells	Diagnosis of heart attack (myocardial infarction [MI])	Expected level is often below the detection limit of the test; pattern of increase and return to low or undetectable levels is the basis for diagnosis of MI
Rheumatoid factor (RF)	Human IgM autoantibody that is increased in autoimmune diseases like rheumatoid arthritis	Ordered as part of an evaluation of joint inflammation and pain to diagnose rheumatoid arthritis	In the absence of autoimmune diseases like rheumatoid arthritis or Sjögren's syndrome, the value is expected to be low — a typical threshold for this test is < 30 U/mL
C-reactive protein (CRP)	CRP is a protein produced in response to infection or inflammatory processes	To evaluate the severity of inflammatory diseases like rheumatoid arthritis or inflammatory bowel disease	< 1 mg/dL < 10 mg/L
High-sensitivity C-reactive protein (hsCRP)	Measures CRP at concentrations seen in absence of illness or flare-ups of inflammatory diseases	Usually measured with a lipid panel to evaluate cardiovascular risk, related to levels of inflammation in arteries associated with atherosclerotic plaque	American Heart Association® considers average risk to be hsCRP of 1.0–3.0 mg/L
B-type natriuretic peptide (BNP) and NT-proBNP	Comes from a protein made in heart cells called proBNP cleaved to form BNP, helps regulate blood volume, and an inactive peptide called NT-proBNP	Tests for either BNP or NT-proBNP are used to detect and evaluate cardiac failure	BNP and NT-proBNP both increase with left ventricle dysfunction; both fall with drug therapy to treat heart failure; either BNP or NT-proBNP can be measured, but the results are not interchangeable
Antistreptolysin O (ASO)	Tests the presence of antibodies to streptolysin O, a toxin made by the group A *Streptococcus* bacteria (*Streptococcus pyogenes*)	Detect a recent streptococcal infection in a patient who has symptoms that may be due to an illness caused by a previous streptococcal infection; it is ordered when symptoms appear	Frequently used cutoff is < 300 IU/L

*Typical values for healthy adults may vary with different patient populations, setting and assay techniques/methodologies. Each lab must verify the range it uses.

**American Diabetes Association® bases its reference intervals on the results of the Diabetes Control and Complications Trial (DCCT) and the values standardized to the National Glycohemoglobin Standardization Program (NGSP). The International Federation of Clinical Chemistry (IFCC) recommends using reference intervals in mmol of hemoglobin A1c per mole of hemoglobin based on its standardization program.

***American Diabetes Association. *2. Classification and diagnosis of diabetes: Standards of Medical Care in Diabetes—2020. Diabetes Care*. 2020;43 (suppl 1):S14-S31.

LIPIDS AND LIPOPROTEINS

Lipids and lipoproteins are measured primarily as an indicator of risk of cardiovascular disease. Interpretation of risk is based on a number of different lipids. Some of the analytes in the lipid risk profile may be elevated as a result of other underlying diseases like hypothyroidism, diabetes or kidney disease. It is important to rule out these possible causes of lipid abnormalities before treating these solely as cardiovascular risk factors.

Lipids and lipoproteins

Analyte	Description	Why measured	Typical reference intervals for healthy adults*	Reasons for increased and decreased values
Total cholesterol	Important steroid lipid, made by the liver and used for production of steroid hormones and cell walls	High cholesterol has been implicated as a risk factor for coronary artery disease	< 200 mg/dL < 5.18 mmol/L	↑ Hypothyroidism, uncontrolled diabetes, kidney disease ↓ Liver diseases, starvation, anemia
High density lipoprotein (HDL) cholesterol	HDL removes excess cholesterol from tissue for disposal; elevated HDL has been found to protect against coronary artery disease	Part of cardiovascular risk profile	Male > 37 mg/dL > 0.96 mmol/L Female > 40 mg/dL > 1.04 mmol/L	↑ Estrogen therapy, alcohol consumption ↓ Smoking
Low density lipoprotein (LDL) cholesterol	LDL carries cholesterol from the liver to peripheral tissue; contributes to formation of plaques that clog arteries and lead to coronary heart disease	Part of cardiovascular risk profile	< 100 mg/dL** < 2.6 mmol/L**	↑ High saturated fat diets, inherited disorders of cholesterol metabolism ↓ High fiber intake, drug treatment

*Typical values for healthy adults may vary with different patient populations, setting and assay techniques/methodologies. Each lab must verify the range it uses.

**AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation*. 2019;139:e1082-e1143. doi:10.1161/CIR.0000000000000625

Lipids and lipoproteins

Analyte	Description	Why measured	Typical reference intervals for healthy adults*	Reasons for increased and decreased values
Very low density lipoprotein (VLDL) cholesterol	Triglyceride-rich lipoprotein that is secreted by the liver and is the precursor to LDL	Part of cardiovascular risk profile	< 30 mg/dL < 0.77 mmol/L	↑ High saturated fat diets, inherited disorders of cholesterol metabolism ↓ High fiber intake, drug treatment
Triglycerides	Chemical form of fatty acids for transport and storage in adipose tissue	Part of cardiovascular risk profile	< 150 mg/dL** < 1.7 mmol/L**	↑ Hypothyroidism, alcoholism, liver disease, uncontrolled diabetes
Lipoprotein(a) Lp(a)	Variant form of LDL that has extra protein chain attached	High Lp(a) is associated with higher risk of cardiovascular disease	< 30 mg/dL 1.07 umol/L 28%–53% of total lipoproteins	↑ Inherited trait
Apolipoprotein A1	Protein portion of HDL	Sometimes included in cardiac risk profiles	Male 94–178 mg/dL 0.94–1.78 g/L Female 101–199 mg/dL 1.01–1.99 g/L	↑ Estrogen therapy, alcohol consumption ↓ Smoking
Apolipoprotein B	Protein portion of VLDL and LDL	Sometimes included in cardiac risk profiles	Male 63–133 mg/dL 0.63–1.33 g/L Female 60–126 mg/dL 0.60–1.26 g/L	↑ High saturated fat diets, inherited disorders of cholesterol metabolism ↓ High fiber intake, drug treatment

*Typical values for healthy adults may vary with different patient populations, setting and assay techniques/methodologies. Each lab must verify the range it uses.

**Mikolasevic, L; Zutelija, M; Mavrinac, V; Orlic, L. Dyslipidemia in patients with chronic kidney disease: etiology and management. *International Journal of Nephrology and Renovascular Disease*. 7 Feb. 2017.

THERAPEUTIC DRUG MONITORING

In general, most prescribed drugs do not require any special monitoring of the drug level in blood, although occasionally they do require tests to ensure that the drug is not adversely affecting liver or kidney function.

The drugs that do require monitoring of their blood concentration are those that have a narrow therapeutic window. This means that there is a very narrowly defined concentration at which the drug is active and effective, but not toxic. If the drug level falls below the lower limit, the drug is ineffective. If it rises above the upper limit, the patient is at risk of health issues due to toxicity.

Ensuring that the patient is receiving the appropriate treatment is a challenge when using drugs with narrow therapeutic windows like some antibiotics. The laboratory is frequently called upon to test drug concentrations at times when the concentration is expected to reach a maximum to assess for risk of toxicity, and again when the drug is expected to reach a minimum concentration, usually immediately before the next dose, to ensure minimum therapeutically effective amounts are maintained. These two times of measurement are referred to as peak and trough concentrations, respectively. Most therapeutic drugs monitor trough concentrations with the exception of some antibiotics with high risk of toxicity (where peak and trough concentrations are medically necessary).

Therapeutic drug monitoring

Analyte	Purpose	Therapeutic range*
Amikacin	Antibiotic	Peak: 25–35 µg/L (43–60 µmol/L) Trough: 1–8 µg/L (6.8–13.7 µmol/L)
Carbamazepine	Control of seizures	4-12 µg/mL (17–51 µmol/L)
Digoxin	Treatment of chronic atrial fibrillation and heart failure	0.8–2 ng/mL (1–2.6 nmol/L)
Gentamicin	Antibiotic	Peak: 5–10 µg/mL (10.5–20.9 µmol/L) Trough: < 1–4 µg/mL (< 2.1–8.4 µmol/L)
Lithium	Treatment of manic-depressive disorders	0.6–1.2 mmol/L
Phenobarbital**	Used for sedation and epilepsy treatment	15–40 µg/mL (65–170 µmol/L)
Phenytoin	Treatment of ventricular arrhythmias and seizures	10–20 µg/mL (40–79 µmol/L)
Quinidine	Prevention of cardiac arrhythmias	2–5 µg/mL (6.2–15.4 µmol/L)
Theophylline	Asthma treatment	8–20 µg/mL (44–111 µmol/L)
Valproic acid	Seizure treatment	50–100 µg/mL (346–693 µmol/L)
Vancomycin	Antibiotic used to treat infections that may be resistant to other antibiotics	Peak: 20–40 mg/L (14–28 µmol/L) Trough: 5–10 mg/L (3v7 µmol/L)

*Typical values may vary with different patient populations, setting and assay techniques/methodologies. Each lab must verify the range it uses.

**Reference interval source from *Clinical Chemistry: Theory, Analysis, and Correlation*, 5th edition.

TOXICOLOGY AND DRUGS OF ABUSE

Drug overdoses and drug toxicities are commonly seen in emergency medicine settings. The laboratory is called upon to help determine if a drug overdose might be responsible for the symptoms a patient is experiencing.

In addition to testing for medical treatment, laboratories are sometimes called upon to test for use of illegal substances. Most often this type of testing, done for employment screening or for legal prosecution, is known as forensic (legal) testing. Labs that perform these tests are specially equipped and licensed to handle samples accompanied by what is termed a “chain of custody.” Chain of custody ensures that the results will be acceptable in a court of law. Most routine clinical chemistry laboratories do not perform this kind of medicolegal testing.

OVERDOSES

Many substances that are readily available and in common use can be toxic if consumed in quantities that exceed the body’s capability to metabolize them. Some analytes that laboratories are frequently called upon to measure are included in the table below.

Toxicology

Analyte	Description	Expected values*
Acetaminophen/Paracetamol (Tylenol®)	Relieves pain and fever	Therapeutic 10–30 µg/mL (66–199 µmol/L) Toxic > 200 µg/mL (> 1,324 µmol/L)
Salicylate (aspirin)	Relieves pain and fever	Therapeutic 150–300 µg/dL (1.09–2.17 µmol/L) Toxic > 500 µg/dL (> 3.62 µmol/L)
Ethanol (alcohol)	Metabolic depressant	Impairment 50–100 mg/dL (11–22 mmol/L) Depression of CNS > 100 mg/dL (> 21.7 mmol/L) Fatalities reported > 400 mg/dL (> 86.8 mmol/L)

*Typical values for healthy adults may vary with different patient populations, setting and assay techniques/methodologies. Each lab must verify the range it uses.

DRUGS OF ABUSE

Most drug abuse testing is done using urine samples in a qualitative manner. A positive test indicates the presence of a drug above a particular level (cutoff). Additional testing is performed on the positive samples using more specific quantitative methods (e.g., gas chromatography/mass spectrometry) to confirm the presence of the drug.

Toxicology

Analyte	Description	Typical cutoffs for a positive test in urine
Amphetamines	Central nervous system stimulants (uppers)	500 ng/mL or 1,000 ng/mL
Barbiturates	Sedatives and hypnotics	200 ng/mL
Benzodiazepines	Antianxiety agents	200 ng/mL
Cannabinoids (marijuana)	Hallucinogen	50 ng/mL or 100 ng/mL
Cocaine	Stimulant	150 ng/mL or 300 ng/mL
Ecstasy	Stimulant	500 ng/mL
Methadone	Analgesic for severe pain and for treatment with opiate addiction	300 ng/mL
Opiates	Analgesic for moderate pain	300 ng/mL or 2,000 ng/mL
Phencyclidine (PCP)	Hallucinogen	25 ng/mL

REVIEW QUESTIONS: SECTION 6

Answers are provided at the end of this Learning Guide.

1. Which of the following tests is a good marker of nutritional status?

   A. Immunoglobulin M

   B. Prealbumin

   C. Ceruloplasmin

   D. Lp(a)

2. Which test might be used to assess a person who is disoriented or confused?

   A. Cholesterol

   B. Ammonia

   C. CRPs

   D. Iron

3. Which tests might be ordered on a patient with abdominal pain to test for possible pancreatitis?

   A. Amylase and lipase

   B. Sodium and potassium

   C. Cholesterol and triglyceride

   D. C3 and C4

4. Which of these drug levels would be considered toxic?

   A. Alcohol at 80 mg/dL

   B. Valproic acid at 50 µg/mL

   C. Digoxin at 2 ng/mL

   D. Acetaminophen at 250 µg/mL

   E. Salicylate at 27 mg/dL

5. Which test is used as an indicator of congestive heart failure?

   A. CRP

   B. BNP

   C. Cholesterol

   D. Troponin

   E. Haptoglobin

SECTION 7

TESTING IN CLINICAL PRACTICE

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LEARNING OBJECTIVES

After completing this section, you will be able to:

1. Identify the tests that are used to diagnose and monitor diabetic patients.
2. Describe the use of lipid tests in assessing the risk of cardiovascular disease (CVD).
3. Identify the roles of the thyroid stimulating hormone (TSH), thyroxine (T4) and triiodothyronine (T3) tests in evaluating thyroid dysfunction.
4. Explain the patterns of test results seen in assessment of anemias.
5. Describe the tests used to assess kidney function.

OVERVIEW

This section reviews laboratory testing used in five areas of clinical practice — diabetes management, heart disease, thyroid disorders, iron deficiency and kidney function.

KEY CONCEPTS

1. A single laboratory analyte may sometimes be used for screening, for definitive diagnosis and to monitor therapy or disease progression.
2. Combinations of tests are often necessary to establish a diagnosis.
3. A single test may not be sufficient for diagnosis; therefore, patterns of test results can be important discriminators.

This section will look at five clinical conditions that rely heavily on clinical chemistry tests for identification of the condition, assessing treatment progress and detecting possible treatment side effects. In some cases, the marker is only available on a different instrument system.

The reader should consult Appendix B: References for more detailed information about these topics, especially *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*, 7th edition, 2015; the Lab Tests Online® website (www.labtestsonline.org); the American Diabetes Association® (ADA) website (www.diabetes.org); the National Cholesterol Education Program (NCEP) website (www.nhlbi.nih.gov/about/ncep); and the National Kidney Foundation® (NKF®) website (www.kidney.org/kidneydisease/ckd/).

DIABETES

Diabetes mellitus is the impaired ability to make or use insulin, the hormone that stimulates uptake of glucose by cells. When insulin's action is impaired, blood glucose is inappropriately high.

- Type 1 diabetes is caused by impaired insulin production.
- Type 2 diabetes is caused by resistance to the action of insulin at the cellular level.
- Gestational diabetes occurs during pregnancy and, like type 2, reflects insulin resistance. Gestational diabetes is transient, disappearing after birth, although there is some evidence that women manifesting gestational diabetes during pregnancy are at risk for developing type 2 diabetes later in life. Infants born to mothers who have gestational diabetes are also at risk for developing diabetes.

SCREENING AND DIAGNOSTIC TESTS

In early stages, diabetes has no obvious symptoms. Glucose screening tests are needed to identify high glucose concentrations in otherwise asymptomatic people. These tests may be done using a fingerstick sample and portable glucose meter, as is typical at health fairs, or using a blood sample drawn by venipuncture and measured in a laboratory.

The guidelines for interpretation of screening tests for blood glucose are shown in the table below. Diagnosis of diabetes is based on fasting blood glucose. Sometimes screening may be done when the person is not fasting. In such cases, interpretation is difficult; however, a non-fasting blood glucose above 200 mg/dL (11.1 mmol/L) is considered to be consistent with diabetes.

Fasting blood glucose	
From 70 to 99 mg/dL (3.9 to 5.5 mmol/L)	Nondiabetic fasting glucose (normal)
From 100 to 125 mg/dL (5.6 to 6.9 mmol/L)	Impaired fasting glucose (prediabetes)
126 mg/dL (7.0 mmol/L) and above on more than one testing occasion	Diabetes

In some circumstances, when a fasting glucose test gives a high result, an oral glucose tolerance test may be ordered. However, since the development of newer HbA1c assays that are designed to permit the diagnosis of diabetes, OGTT is no longer used frequently. The HbA1c test doesn't require any patient preparation or fasting, whereas the OGTT requires fasting and additional glucose challenges, as outlined below. As a result of this combination of factors, HbA1c has largely displaced the OGTT.

There are several different versions of the oral glucose tolerance test (OGTT). All involve fasting for a minimum of 8 hours, followed by ingestion of a fixed amount of glucose and one or more tests for blood glucose at specified times following ingestion.

A 75-gram test is sometimes used for adults suspected of having diabetes. However, glucose tolerance tests are most often used to diagnose gestational diabetes. A 50-gram challenge test is sometimes ordered as a screening test to follow up an abnormal fasting value. If this is abnormal, the 50-gram screening test may be followed by a more definitive OGTT with a 100-gram glucose test.

Adult oral glucose tolerance test (OGTT) (2 hours after a 75-gram glucose drink is consumed)	
Less than 140 mg/dL (7.8 mmol/L)	Normal glucose tolerance
From 140–200 mg/dL (7.8–11.1 mmol/L)	Impaired glucose tolerance (prediabetes)
Over 200 mg/dL (11.1 mmol/L) on more than one testing occasion	Diabetes
Initial screening OGTT for gestational diabetes (50-gram glucose drink)	
1-hour sample	< 140 mg/dL (7.8 mmol/L) is normal
Diagnostic OGTT for gestational diabetes (100-gram glucose drink)	
Fasting	95 mg/dL (5.3 mmol/L)
1 hour after glucose load	180 mg/dL (10.0 mmol/L)
2 hours after glucose load	155 mg/dL (8.6 mmol/L)
3 hours after glucose load	140 mg/dL (7.8 mmol/L)

MONITORING OF DIABETES AND DIABETIC COMPLICATIONS

A patient with diabetes is at risk for developing a number of complications that are a direct consequence of high levels of glucose. These include kidney failure, blindness, poor circulation leading to foot ulcers, and an increased risk for atherosclerosis and heart disease.

Treatment of diabetes with diet, drugs and insulin is aimed at maintaining blood glucose levels as close to nondiabetic levels as possible. Research studies, including the Diabetes Control and Complications Trial (DCCT), have demonstrated that good control of blood glucose can slow or prevent the development of the many complications that accompany poorly controlled blood glucose.

Diabetic patients often monitor their own blood glucose level regularly to ensure that their diet and medications are appropriate to keep their blood glucose within a target range set by their doctor.

Two other very important tests that are used to confirm glucose control and ensure good kidney function are hemoglobin A1c and urine albumin (also known as microalbumin).

HEMOGLOBIN A1c (HbA1c)

Hemoglobin A1c (HbA1c) is a chemically modified hemoglobin molecule. It forms when glucose from the blood enters the red blood cells and attaches to hemoglobin. As the glucose concentration in blood increases, more glucose reacts with hemoglobin. Since red blood cells circulate with a half-life of 3 months, meaning that half of the red blood cells are destroyed and replaced by new ones every 3 months, the extent to which hemoglobin has been altered by glucose reflects glucose control over the previous 3 months. Hemoglobin A1c is expressed as a percent that reflects the percentage of hemoglobin molecules that have a glucose molecule attached. HbA1c can now be used to diagnose and/or screen for diabetes.

URINE ALBUMIN (MICROALBUMIN)

Urine albumin (microalbumin) is a test for very small amounts of albumin escaping from the kidney and leaking into the urine. The first sign of albumin in the urine is a signal that kidney function is being compromised. Early detection can lead to more aggressive treatment to prevent continuing damage to the kidney.

Test	Frequency	Why done
Blood glucose	Daily (by patient)	To monitor glucose control and adjust medications to maintain target blood glucose level
HbA1c (hemoglobin A1c, glycohemoglobin, glycated hemoglobin)	2–4 times per year	Reflects glucose control over a 3-month period. May be used to diagnose or screen for diabetes
Urine albumin (microalbumin)	1–2 times per year	For early identification of decreasing kidney function

http://professional.diabetes.org/GlucoseCalculator.aspx.

Lipid profiles are often included as part of diabetes care since diabetics are at increased risk of developing CVD. The next section discusses these tests.

CARDIOVASCULAR DISEASE

Cardiovascular diseases are the most common causes of death worldwide, and they affect individuals from all backgrounds, incomes and nations. Of the estimated 17 million deaths attributed to cardiovascular disease worldwide in 2015, the World Health Organization estimates that 7.4 million were due to coronary heart disease, which is a disease of the coronary arteries.[1] Coronary heart disease manifests most often in the form of **acute coronary syndrome** (ACS), the general term used to describe a myocardial infarction (the medical term for heart attack) and unstable angina (the medical term for chest pain from heart disease). The American Heart Association® estimates that, in the United States, someone experiences a myocardial infarction every 42 seconds.[2] Of these individuals, approximately 15 percent will die within 1 year as a result of the myocardial infarction.[2] Similarly, in Europe, coronary heart disease is the single leading cause of mortality and the most common cause of premature death (before the age of 65 years).[3] Despite significant medical progress in diagnosing and treating cardiovascular diseases, the World Health Organization estimates that it will still be the most common cause of death worldwide in 2030.[4] **Heart failure** is also a major cause of morbidity and mortality. It is estimated that over 26 million people worldwide are living with heart failure, and it is the number one cause of hospitalization in the United States and Europe.[14] In the United States, it is estimated that over 6.5 million adults are living with heart failure,[14] and that figure is predicted to grow to over 8 million by 2030.[15] Registries of heart failure patients in the United States and Europe report mortality to be anywhere from 23% to 36% during the first year after a heart failure hospitalization. Circulating biomarkers play an important role in many aspects of cardiovascular disease.

ACUTE CORONARY SYNDROME AND MYOCARDIAL INFARCTION

Acute coronary syndrome is a general name for a group of conditions that indicate that the heart muscle is not receiving enough oxygen. This situation is termed myocardial ischemia. There are three subcategories of ACS: ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI) and unstable angina (UA).

In ACS, paramedics, nurses and physicians use the ECG to differentiate a STEMI from other causes of ACS symptoms. An ECG can even identify which coronary artery is likely obstructed by looking at which leads have ST elevation.

ST-ELEVATION MYOCARDIAL INFARCTION

An ST-elevation myocardial infarction (STEMI) is often the most severe form of ACS, and it is a type of myocardial infarction (MI). It is estimated that STEMI accounts for about 25% of all MI, but it carries a high risk of mortality, with 10% of patients dying in the first 30 days after the event.[2]

NON-ST-ELEVATION MYOCARDIAL INFARCTION

The second type of ACS, non-ST-elevation myocardial infarction or NSTEMI, also has serious consequences for the heart. An NSTEMI is also a type of MI but is much more common than STEMI. Although estimates vary, approximately 75% of patients presenting with MI are diagnosed with NSTEMI, and this is associated with an 18% chance of death in the first 30 days after the event.[2]

UNSTABLE ANGINA

The third type of ACS is unstable angina (UA). Unlike STEMI and NSTEMI, traditional cardiac biomarkers are not elevated in UA.[5,6]

The key concept to appreciate about MI is that anything that prevents the myocardium from receiving adequate supplies of oxygen will lead to ischemia. If this ischemia is prolonged, it will lead to cardiac myocyte (i.e., cardiac muscle cell) death and tissue necrosis. Because damage to the cardiac tissue happens quickly once an obstruction occurs, it is essential that medical providers understand how to manage patients experiencing ACS efficiently. Any patient with ACS symptoms is classified as very high acuity in the emergency department and requires rapid assessment and close monitoring. Nurses, physicians and other support staff must work together to ensure that this occurs in a timely and efficient manner.

Although the assessment process for acute MI begins with an ECG, clinicians must also consider the symptoms the patient is experiencing and measure cardiac biomarkers. Cardiac troponins are the preferred biomarker in this clinical setting.

TROPONINS IN THE DIAGNOSIS OF MYOCARDIAL INFARCTION

The cells of the myocardium contain three types of troponin: troponins I, T and C. Troponin C is also found in skeletal muscle, but troponins I and T are very specific to cardiac myocytes and are therefore clinically useful for measuring damage to the myocardium. When extended ischemia occurs, the cells die and begin to break apart, releasing the troponin into the bloodstream.[5] If high levels of troponin T or I are measured in the blood, in the correct clinical setting, it is confirmation that there is necrosis in the cardiac tissue.[5] To be diagnostic for MI, cardiac troponin must not only exceed the 99th percentile of the upper reference limit, but also must be rising or falling in a characteristic pattern over time. Serial measurements of troponin can determine if concentrations are changing; when troponin concentrations remain constant over an extended duration, it suggests that an alternative diagnosis should be considered, such as renal failure, heart failure or sepsis.[8]

Notably, most healthy individuals have small but measurable concentrations of troponin in the bloodstream in a normal physiologic state, possibly as a result of cell turnover (the normal life and death cycle of a cell).[9] However, at these low concentrations, only high-sensitivity assays can detect it.[9]

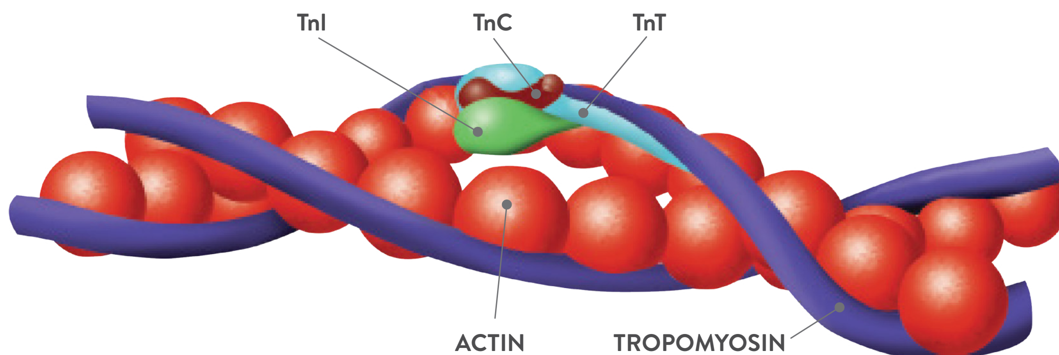


Figure 7-1: The troponin complex.

HIGH-SENSITIVITY TROPONINS

Due to the shortcomings of conventional troponin measurements, high-sensitivity troponin (hsTn) assays have been developed. The hsTn assays have been used for several years outside of the United States. These assays are tenfold to hundredfold more sensitive than the conventional troponin assays and can detect the very low levels of troponin that circulate in healthy individuals (**Figure 7-2**).[11] They can also dramatically shorten patient evaluation time in the ED and have demonstrated that they can reduce overall healthcare costs.[37] Furthermore, they avoid the predicament of potentially missing an MI with a lesser degree of troponin elevation.

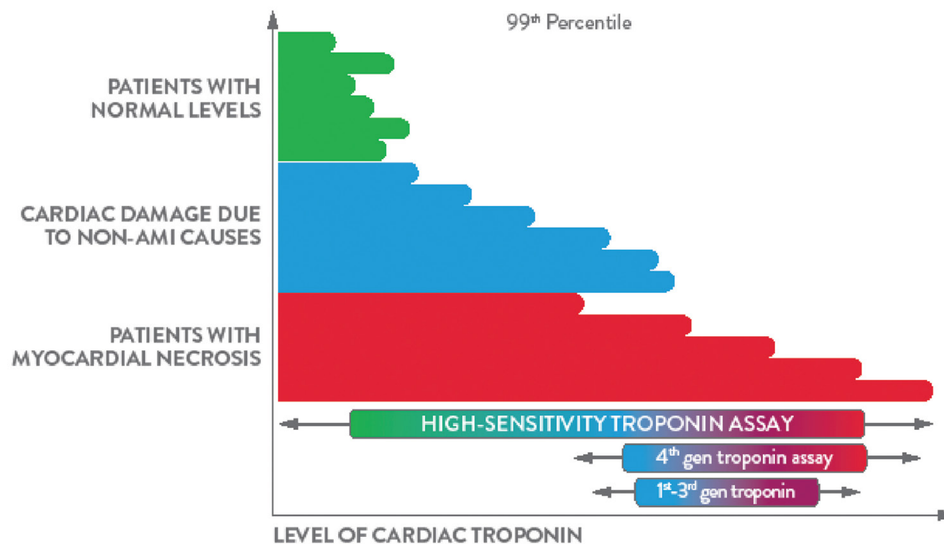


Figure 7-2: The sensitivity of high-sensitivity troponin assays allows for measurement of troponin in normal healthy individuals, whereas earlier generation assays were only sensitive enough to detect troponin elevation resulting from significant necrosis in MI or, occasionally, severe damage from other causes.

Adapted from: Garg P, Morris P, Fazlanie AL, et al. "Cardiac Biomarkers of Acute Coronary Syndrome: from History to High-sensitivity Cardiac Troponin." *Internal and Emergency Medicine*. 2017;12(2):147-155. Used under CC BY http://creativecommons.org/licenses/by/4.0/ modified from original.

HEART FAILURE AND NATRIURETIC PEPTIDES

Heart failure, in the simplest of terms, indicates that a problem with the ventricles is preventing the heart from filling and/or pumping correctly. It is the result of a complex mechanical and neurohumoral syndrome resulting in stasis (or slow movement) of blood in the lungs and peripheral tissues. Heart failure initiates structural, functional and neurohumoral abnormalities that prevent the ventricles from either properly filling with blood or properly ejecting blood. Regardless of the underlying mechanism, this results in poor cardiac performance.

During an initial diagnostic workup for heart failure, a considerable amount of laboratory testing is performed. Renal and liver panels, lipid measurements, thyroid function tests, complete blood counts, and iron studies may be ordered to evaluate for underlying causes and comorbidities and also to assess the appropriateness of heart failure therapies.[17] In addition, several circulating biomarkers are useful in the diagnosis and management of heart failure.

Natriuretic peptides

The natriuretic peptides (NPs), B-type natriuretic peptide (BNP) and N-terminal pro B-type natriuretic peptide (NT-proBNP) play a central role in heart failure. Although BNP was initially called "brain natriuretic peptide" because it was first identified in the brains of pigs, this was a misnomer as it is very specific to the heart.[19] BNP is a hormone released by the cardiac myocytes when they are under strain, and NT-proBNP is an inactive fragment cleaved from the BNP molecule.[20] These NPs are released when the ventricles, particularly the left ventricle, experience volume and pressure overload and when neurohormonal activation occurs in response to heart failure.[20] Measuring NPs has shown benefit in almost every aspect of heart failure care. In the hospital setting, NPs can assist in distinguishing the cause of acute shortness of breath, determine prognostic information about mortality risk in AHF and, when measured at discharge, can identify long-term prognosis after an AHF admission.[17,18,21] They can also be used as a screening tool in the outpatient setting to rule out heart failure or identify patients in need of early intervention to prevent heart failure.[17,18] Finally, they can provide long-term prognostic information for patients with chronic heart failure.[21] Section 5 will discuss the NPs in more detail.

Galectin-3

Galectin-3 is another biomarker that increases with worsening heart failure. Galectin-3 concentrations appear to increase when fibroblasts and macrophages are activated in the cardiac tissue.[19,21] These cells are involved in the remodeling of cardiac muscle that occurs in heart failure. Consequently, galectin-3 appears to be a good marker for the presence of cardiac remodeling and the development of cardiac fibrosis. In clinical studies, galectin-3 has demonstrated usefulness as a prognostic indicator and as a screening tool for assessing readmission risk after a heart failure hospitalization.[22,23]

OTHER CARDIAC BIOMARKERS

Although troponin is central in the determination of myocardial necrosis, a variety of other markers have demonstrated some usefulness in the evaluation of patients with suspected ACS in the past, and more are under investigation. Myoglobin, an oxygen-carrying protein, was one of the earliest biomarkers used for MI.[9] Although its concentrations rise in response to injury of the cardiac tissue, it is no longer recommended as a biomarker for ACS.[9]

Creatinine kinase MB (CK-MB) is a marker that is mostly specific to the cardiac tissue and was traditionally used in conjunction with older generation troponin assays. It remains elevated for a shorter time than troponin after MI, it may provide additional clinical information regarding the timing of a myocardial injury and it is sometimes useful for detecting an early reinfarction.[10] In current practice, routine measurements of CK-MB in patients with suspected ACS are no longer recommended.[10]

CARDIOVASCULAR RISK BIOMARKERS

LIPIDS

Circulating lipids, often referred to as cholesterol, are essential building blocks for plaque in atherosclerotic disease. As such, lipid measurements are useful as predictors of cardiovascular disease and were some of the earliest circulating biomarkers used for assessing cardiovascular risk. Moreover, when medications, specifically statins, are employed to normalize certain lipid biomarkers, the rate of cardiovascular events and death is significantly reduced. As such, lipids are a cornerstone of cardiovascular risk assessment and are included in many risk prediction scoring tools.

Total cholesterol

Higher concentrations of total cholesterol have long been associated with an increased risk for CVD.[25,26] Because of this correlation, total cholesterol concentrations are included in many CVD risk assessment tools. Although this measure remains a helpful tool for evaluating risk, it is no longer used as a target for intervention with lipid-lowering medication.

Low-density lipoprotein

Low-density lipoprotein (LDL) is also very strongly correlated with risk of cardiovascular disease; as LDL concentrations increase, so does the risk for cardiovascular events.[24,25,27] Importantly, use of statin medication in patients with elevated LDL concentrations reduces the risk of cardiovascular events and death.[24,27] Both the U.S. and European guidelines for lipid management use LDL as the primary biomarker for determining CVD risk and the need for intervention with medication therapy.[24,27] Of all the lipid markers, LDL is the most established for identifying risk and guiding medication interventions to lower risk of CVD and death.

High-density lipoprotein

High-density lipoprotein (HDL), sometimes referred to as "good cholesterol," is inversely associated with CVD; so, unlike other forms of lipid biomarkers, as HDL increases, the risk of CVD declines.[25,28] The concentrations of HDL are reported in most lipid panels and provide clinicians with additional insight about CVD risk. However, unlike LDL, drug therapies to raise HDL do not appear to reduce the risk of cardiovascular events in clinical trials, and it is unlikely that low HDL itself is a cause of CVD.[29,30] Although lifestyle factors such as exercise and smoking cessation are interventions that can raise HDL and decrease cardiovascular risk, the benefits of these interventions are not entirely due to their effect on HDL.

For more information on cardiovascular disease and related laboratory testing, please see the Cardiac Learning Guide. This guide can be found at corelaboratory.abbott.

THYROID DISEASES

The thyroid gland, a small gland located in the throat, produces two hormones, thyroxine (T4) and triiodothyronine (T3), which control energy metabolism in tissue. Low amounts of circulating T3 and T4 are sensed by the hypothalamus gland, which produces thyroid releasing hormone (TRH) that in turn stimulates the pituitary gland to produce thyroid stimulating hormone (TSH), which stimulates the thyroid gland to produce T3 and T4. This elaborate feedback system ensures appropriate energy metabolism.

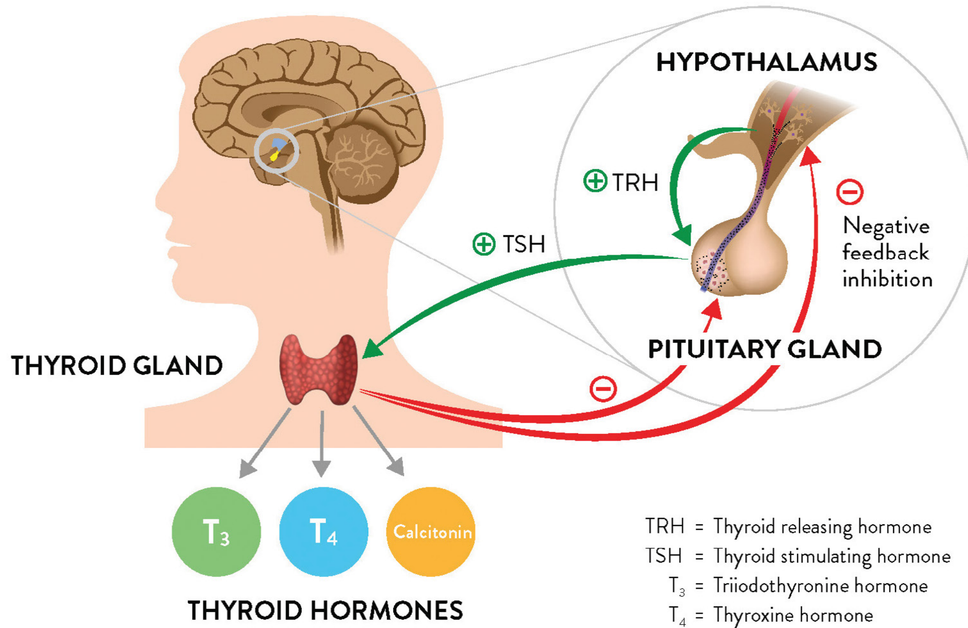
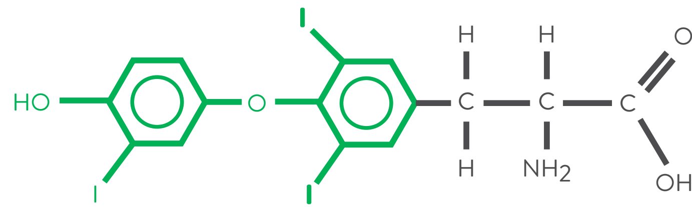
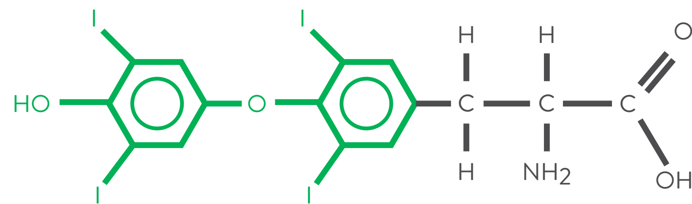


Figure 7-3: Regulation of thyroid function.



3’, 3, 5-Triiodothyronine, T_3



3’, 5’, 3, 5-Tetraiodothyronine
Thyroxine, T_4

Figure 7-4: Thyroid disease.

Hypothyroidism is defined as an underactive thyroid gland. It results in a condition in which the metabolism is slowed down. Hypothyroidism causes symptoms such as fatigue, weight gain, dry skin, hair loss and chills due to poor regulation of body temperature.

Hyperthyroidism is defined as an overactive thyroid gland. It is a condition in which the metabolism is sped up. Hyperthyroidism causes weight loss, increased heart rate, difficulty sleeping and anxiety.

THYROID STIMULATING HORMONE (TSH)

TSH is often measured as a screening test for thyroid function. If TSH is outside the usual reference interval, tests for T4 and/or T3 may be ordered to evaluate thyroid function. Since T4 and T3 both circulate with the majority of the hormone bound to a transport protein (thyroid-binding globulin), a laboratory test may measure the total amount of hormone (total T4 or total T3) or may measure only the unbound or free portion (free T4 and free T3). The free portion is the biologically active portion and is often preferred to the total since the total may be affected by non-thyroid conditions that affect binding protein concentrations. The typical approach to screen for thyroid disease is to perform a TSH test. If it is abnormal, a free T4 is performed.

The following table identifies how these test results are often used to identify possible problems.

TSH	T4 or free T4	T3 or free T3	Interpretation
High	Normal	Normal	Mild (subclinical) hypothyroidism
High	Low	Low or normal	Hypothyroidism
Low	Normal	Normal	Mild (subclinical) hyperthyroidism
Low	High or normal	High or normal	Hyperthyroidism
Low	Low or normal	Low or normal	Nonthyroid illness

TSH testing is also used to monitor treatment of hypothyroidism to ensure the dosage is appropriate.

ANEMIA, IRON AND NUTRITION

Anemia is a condition categorized by a lessened capacity of blood to carry enough oxygen — most commonly due to a lower than normal amount of red blood cells or hemoglobin. Anemias may be caused by a variety of conditions such as:

- Blood loss — Blood loss due to menstruation in women, bleeding from a tumor in the colon, blood donation or intravascular hemolysis depletes iron stores needed to make new red blood cells.
- Dietary deficiency — Either a nutrient-deficient diet or a problem with absorption of iron from the gut may lead to an iron deficiency.
- Inadequate erythropoietin (EPO) — The kidney may fail to produce EPO, an important hormone that stimulates production of red blood cells in the bone marrow.

Tests to evaluate anemias attempt to pinpoint the cause. Numbers and sizes of red blood cells are typically evaluated in the hematology section of the lab as part of screening for anemias. Some of the clinical chemistry tests that are important in evaluation of anemias and iron status include the following.

IRON

Iron is a necessary nutrient that is incorporated into a complex called heme that is the oxygen-binding entity in red blood cells (part of hemoglobin) and muscles (part of myoglobin) as well as a number of important enzymes in various cells and tissues. About 1.2 milligrams of iron are lost each day as a result of breakdown of iron-containing molecules. The lost iron needs to be replaced by dietary iron to maintain sufficient iron for production of heme, hemoglobin, red blood cells and other important molecules.

TRANSFERRIN

Transferrin is a transport protein that carries iron in the circulation. Each molecule of transferrin is capable of carrying two atoms of iron so the total iron-binding capacity (TIBC) is determined by the amount of transferrin present. The numbers of open sites on transferrin that could bind additional iron are referred to as unsaturated iron-binding capacity (UIBC). Both TIBC and UIBC can be measured in the chemistry laboratory.

FERRITIN

Ferritin is a storage protein that binds and stores iron in tissue, mainly the liver. Some ferritin circulates in the blood. The blood level is used as a surrogate marker for the amount of ferritin inside cells.

Disease	Iron	TIBC	UIBC	Ferritin
Iron deficiency	Low	High	High	Low
Chronic illness	Low	Low	Low to normal	Normal to high
Chronic malnutrition	Low	Low	Low to normal	Low to normal
Hemochromatosis or iron overload	High	Low	Low	High

FOLIC ACID (FOLATE) AND VITAMIN B_{12} (COBALAMIN)

Two vitamins, folic acid and B_{12}, are crucial for normal red blood cell formation. If either or both are low, sufficient numbers of red blood cells will not be produced and anemia will develop. In pregnancy, extra quantities of B_{12}, and especially folate, are required by the developing fetus, so pregnant women are advised to consume additional folate. Many foods like breads, grains and cereals are fortified with folate. Tests of blood levels of these two vitamins are often performed to ensure adequate amounts are present. If not, causes for deficiencies will be sought. Some causes include absorptive diseases like celiac disease or lack of intrinsic factor, a protein that promotes absorption of vitamin B_{12} from the gut.

HAPTOGLOBIN

Haptoglobin is a protein that binds heme upon the destruction of heme-containing proteins. When a hemolytic process leads to excessive destruction of red blood cells and hemoglobin, the released heme is bound by haptoglobin. The heme-haptoglobin complex is taken up and destroyed by the liver. This process leads to a decreased level of haptoglobin, which can be an indication of anemia due to a hemolytic process.

EPO

Erythropoietin (EPO) is a hormone that stimulates red blood cell production. EPO is made by the kidney in response to sensing low oxygen transport and a need for more red blood cells. In kidney diseases, EPO production is deficient. A test for EPO can help identify anemias that are the result of insufficient production of EPO by the kidney.

KIDNEY FUNCTION

The kidneys filter the blood to remove waste products and toxins and transfer them into the urine for elimination. When the kidneys are not functioning correctly, two kinds of problems occur:

1. Toxins and waste products that should be filtered from the blood into the urine are not, and are found in high concentrations in the blood. Two examples are creatinine and urea nitrogen (BUN).
2. Blood substances that should not be filtered into the urine, but should be held back by the kidney, are escaping into the urine, resulting in high levels in urine and low levels in the blood. An example is the protein albumin.

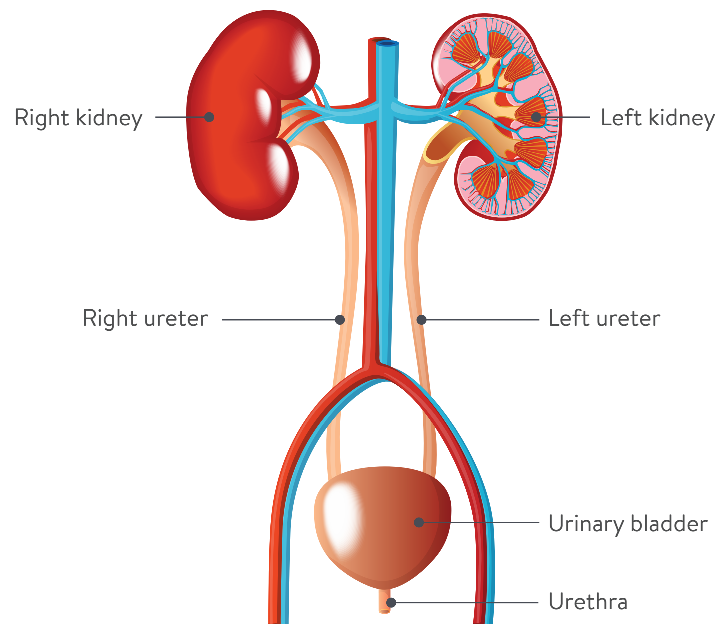


Figure 7-5: Kidney.

The kidney filtration process takes place in structural regions called glomeruli and the filtration is often assessed by a concept called glomerular filtration rate, or GFR. The GFR is expressed as the volume of blood plasma that is cleared of a specified substance each minute. A number of different substances can be used to determine GFR, but the most common is creatinine.

CHRONIC KIDNEY DISEASE (CKD)

Chronic kidney disease is damage to the kidneys over a period of time from diseases such as diabetes or actual physical injury. If kidneys are injured, their normal function of filtering waste is compromised and can lead to kidney failure.

In the early stages of CKD, the symptoms can be unnoticeable, and the majority of patients with CKD will live their lives for years without knowing they have CKD. But as kidney function continues to be compromised, symptoms such as nausea, swelling, increased blood pressure and poor appetite can become more evident.

The good news is that patients with CKD can be identified early with simple lab tests. The complications of CKD can be managed and treated, thus avoiding kidney failure and a lifetime of dialysis and ultimately kidney transplant. CKD can be broken down into five stages. Physicians, with the help of some key laboratory tests, can determine the appropriate stage for the patient.

Gfr and stages of kidney disease

Stage	Description	Glomerular filtration rate (GFR) (mL/min/1.73 m^2)
G1	Kidney damage with normal or increased GFR	≥ 90
G2	Kidney damage with mild decrease in GFR	60–89
G3a	Mild to moderately decreased GFR	45–59
G3b	Moderately to severely decreased GFR	30–44
G4	Severe decrease in GFR	15–29
G5	Kidney failure	< 15 (or dialysis)

National Kidney Foundation®. https://www.kidney.org/es/node/25721#

Common laboratory tests used to assess and diagnose CKD are creatinine, creatinine clearance, GFR, albumin, blood urea nitrogen, calcium, carbon dioxide, chloride, cystatin C, phosphorus, potassium and sodium.

CREATININE CLEARANCE

Creatinine clearance requires a carefully timed urine collection to be able to measure the amount of creatinine excreted over a known time period. This is accompanied by a blood sample taken either at the beginning or end of the urine collection period to measure the plasma concentration.

$$\text{GFR} = \frac{\text{Urine creatinine concentration} \times \text{Rate of urine output in mL/min}}{\text{Plasma creatinine concentration}}$$

If 1 gram of creatinine is excreted into 1.0 liter of urine (1,000 mg/L or 100 mg/dL) in a 24-hour period (1,440 min) and the plasma creatinine concentration is 0.7 mg/dL, the GFR would be:

$$\text{GFR} = \frac{100 \text{ mg/dL} \times 1{,}000 \text{ mL/1,440 min}}{0.7 \text{ mg/dL}} = 99 \text{ mL/min}$$

In adults, GFR can range from 50 to 150 mL/min, with younger people having higher values and older people having lower values.

The collection of an accurately timed urine sample is often problematic. Poorly collected or poorly timed samples may introduce errors into determination of GFR.

ESTIMATED GLOMERULAR FILTRATION RATE (eGFR)

To avoid issues with collection of a timed urine sample, the GFR is estimated using an empirically derived formula. There are a variety of different formulas that estimate GFR from a measured plasma creatinine. These formulas vary in how they take into account factors like age, body size, gender and race. No single formula has been universally adopted. The estimated value from this calculation is designated eGFR for estimated GFR.

GFR can be accurately measured by testing 24-hour urine samples for various endogenous and exogenous markers that are freely filtered by the glomerulus. Measured GFR, however, is difficult and not commonly performed in clinical laboratories. The eGFR is a means to identify patients before kidney function is severely impaired so that therapy can be started to avoid end-stage renal disease (ESRD), which requires renal dialysis or kidney transplant. The eGFR is not as accurate as measured GFR, perhaps approximating +/- 30% of the true value, but it is much more convenient and easy to calculate. The older Cockcroft-Gault formula, $\mathbf{C_{Cr} = ([140 - age] \times weight)/(72\ S_{Cr}) \times 0.85\ (if\ female)}$, dates from the 1970s and is still used for estimating drug dosage by pharmacists. It has been replaced by the MDRD (Modification of Diet in Renal Disease) formula:

$$\mathbf{eGFR = 175 \times (S_{Cr})^{-1.154} \times (age)^{-0.203} \times (0.742\ \text{if female}) \times (1.210\ \text{if black})}$$

The original MDRD eGFR formula uses the factor “186” instead of “175” and was based on a creatinine assay lacking optimal standardization. Most current creatinine assays have been restandardized and are traceable to a new serum-based secondary reference material (SRM 967) and a gold standard reference method, LC-IDMS (liquid chromatography isotope dilution mass spectrometry). It is important to know how the creatinine assay used by a laboratory is standardized to ensure that the correct version of the MDRD equation is used for eGFR. Gender of patients is typically known so the correction factor for females (who have less muscle mass than males and lower creatinine values) can be used as appropriate. The ethnicity of patients is not always known or clear, and it is recommended that two eGFRs, one with and one without the correction for black ethnicity, be reported so that the physician can pick the one more suitable for a given patient. The black ethnicity factor is used because, in general, blacks have larger muscle mass and higher creatinine values than Caucasians. Studies are ongoing to refine the MDRD equation to make it suitable for pediatric and geriatric patients, and other eGFR equations have also been suggested. The MDRD equation is applicable to adults 18–70 years of age.

Some recent studies have indicated that calculating eGFR with cystatin C in place of creatinine or in addition to creatinine is more accurate for certain patient populations like the elderly or children where creatinine values can be impacted by age and sex. Cystatin C appears to be less affected by age and sex and is a good predictor of kidney filtration.

REVIEW QUESTIONS: SECTION 7

Answers are provided at the end of this Learning Guide.

1. Which of the following tests is the best monitor of diabetic glucose control over an 8–12-week period?

   A. Glucose

   B. Urine microalbumin

   C. Hemoglobin A1c

   D. Haptoglobin

2. The lipoprotein particle that is used to determine increased risk of coronary artery disease and to determine and monitor treatment for high cholesterol is:

   A. HDL

   B. LDL

   C. Apolipoprotein A1

   D. Chylomicrons

3. Which test is the most specific for myocardial infarction?

   A. LDH

   B. CK

   C. Troponin

   D. Myoglobin

4. If a screening TSH is high, which test is likely to be ordered next?

   A. Cholesterol

   B. Free T4

   C. Ferritin

   D. Glucose

5. In which condition would TIBC be high?

   A. Hemochromatosis

   B. Chronic illness

   C. Malnutrition

   D. Iron deficiency

6. When the kidneys are not functioning properly to filter blood and rid the body of wastes, which of these test results would be most likely?

   A. GFR = 100 mL/min

   B. High blood creatinine

   C. High blood albumin

   D. Low blood BUN

SECTION 8

UNITS OF MEASURE

KEY CONCEPTS 114

MOLES VERSUS MASS 116

ENZYMATIC ACTIVITY 117

ANALYTES THAT CANNOT BE EXPRESSED IN TERMS OF MOLECULES OR MOLES 118

REVIEW QUESTIONS: SECTION 8 119

LEARNING OBJECTIVES

After completing this section, you will be able to:

1. Identify different types of units for reporting analyte concentrations.
2. Be able to convert units from conventional to Système International (SI).

KEY CONCEPTS

1. Concentrations are measured in amount of substance per volume of solution.
2. Concentrations may be based on mass, number of molecules or activity.
3. Laboratories typically use one of two metric conventions in reporting concentrations of analytes.

For quantitative concentration measurements, such as are typically done in a clinical chemistry laboratory, the results are expressed as numerical values and units.

Consider this example: Cholesterol 192 mg/dL

The numeric value, 192 mg in the example, represents the amount of substance (cholesterol). The volume unit, deciliter (dL) in the example, identifies the amount of fluid containing the substance. Some other important units include the duration of time for sample collection, the path length of the cuvette used for optical measurements, and the temperature at which the analysis is conducted.

Qualitative tests, although reported without units, are based on a threshold value that is defined by a concentration. Positive test results are reported for those samples with the concentration of analyte equal to or above the threshold value. Negative test results are reported for those samples with the analyte concentration below the threshold value. Drug of abuse assays are an example of this type of test.

The reader should consult Appendix B: References for more detailed information about these topics, especially *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*, 7th edition, 2015, and the International Bureau of Weights and Measures (BIPM®) website (www.bipm.org).

The units in clinical chemistry are traditionally metric units. The table below identifies some units that are commonly used in computing or reporting laboratory results.

Type of measure	Basic unit (abbreviation)	Other measures of the basic unit (abbreviation)	Relation to the basic unit
Mass of analyte or test solution	Gram (g)	Kilogram (kg) Milligram (mg) Microgram (μg) Nanogram (ng)	1,000 grams 0.001 or 10^{-3} grams 10^{-6} grams 10^{-9} grams
Molecules of analyte	Mole (mol) [One mole is defined as 6.02×10^{23} molecules]	Millimoles (mmol) Micromoles (μmol) Nanomoles (nmol)	0.001 or 10^{-3} moles 10^{-6} moles 10^{-9} moles
Volume of solution	Liter (L)	Deciliter (dL) Milliliter (mL) Microliter (μL)	0.1 or 10^{-1} liters 10^{-3} liters 10^{-6} liters
Time	Hour (hr)	Minute (min) Second (sec)	1/60th of an hour 1/60th of a minute
Enzymatic activity	International unit (IU) Katal (kat)	MilliIU (mIU) MicroIU (μIU) Millikat (mkat) Microkat (μkat)	10^{-3} IU 10^{-6} IU 10^{-3} kat 10^{-6} kat
Temperature	Degree centigrade (°C)		
Length	Meter (m)	Centimeter (cm) Millimeter (mm) Micrometer (μM)	10^{-2} meters 10^{-3} meters 10^{-6} meters

Two different systems of units are in common use. In the United States, the most frequently used result reporting system employs what are called conventional units. Internationally, most other countries use a convention called the Système International, or SI, units.

MOLES VERSUS MASS

For some common analytes, the two conventions differ in the choice of mass versus moles to express the amount of material. In such cases, the two unit systems can easily be converted using the molecular weight of the analyte. Moles of analyte multiplied by the molecular weight gives grams. Grams of analyte divided by the molecular weight gives moles. Conversion factors are based on the molecular weight and an appropriate multiplier (some factor of 10) to adjust the units of the analyte and reference volume.

An example of converting glucose conventional units (mg/dL) to SI (mmol/L) is shown below. The molecular weight is used to convert mg to mmol, and a factor of 10 is needed to convert the concentration in a deciliter to that in a liter.

$$\frac{\text{100 mg glucose}}{\text{dL}} \times \frac{\text{1 mole}}{\text{180 grams (MW of glucose)}} = \frac{\text{0.55 millimoles of glucose}}{\text{dL}}$$

$$\frac{\text{0.55 mmol of glucose}}{\text{dL}} \times \frac{\text{10 dL}}{\text{L}} = \frac{\text{5.5 mmol of glucose}}{\text{L}}$$

$$\text{Therefore, the conversion factor is } \frac{\text{1 gm}}{\text{180 gm/mole}} \times \frac{\text{10 dL}}{\text{L}} = 0.056$$

The following table lists some examples of tests where conventional and SI units differ because of the use of mass or moles to report analyte concentration.

Test analyte	Conventional units	SI units	Molecular or atomic weight	Conversion formula from conventional to SI units
Bilirubin	mg/dL	μmol/L	585	17.1 × mg/dL = μmol/L
Calcium	mg/dL	mmol/L	40	0.25 × mg/dL = mmol/L
Cholesterol	mg/dL	mmol/L	386	0.0259 × mg/dL = mmol/L
Creatinine	mg/dL	μmol/L	113	88.4 × mg/dL = μmol/L
Glucose	mg/dL	mmol/L	180	0.056 × mg/dL = mmol/L

ENZYMATIC ACTIVITY

Enzymes are catalysts that speed up chemical reactions. Enzymatic activity is a reflection of how fast the reaction goes in the presence of an enzyme. The lower the amount of enzyme present, the slower the reaction. The higher the amount of enzyme present, the faster the reaction. If the same reaction goes twice as fast using serum from one patient as serum from another, the first patient is said to have twice the enzymatic activity in his blood as the other patient.

Enzymatic activity, which is expressed in terms of the rate of a catalyzed chemical reaction, is measured as number of moles of the starting chemical compound (called a substrate) converted to product in a given time (per second or per minute).

In the two reporting conventions, different options are available to express the rate of conversion of substrate.

Conventional	Enzyme unit (U)	μmol/min
SI	Katal (kat)	mol/sec

Conversion of: 1 μmol/min to mol/sec

$$\frac{1\ \mu mol}{min} \times \frac{1\ mol}{10^6\ \mu mol} \times \frac{1\ min}{60\ sec} = 1.7 \times 10^{-8}\ \text{mol/sec or kat or } 0.017\ \mu kat$$

If an enzyme is able to act on a variety of different substrates, converting them to different chemical forms, any one of those substrates can be used to test for the enzyme's activity. The reaction conditions (such as temperature and pH) used to measure the substrate conversion to product will affect the conversion rate. Higher temperatures typically result in faster reactions. Changes in pH may affect the enzymatic activity, with a particular pH promoting optimum activity and others resulting in slower reactions. So reporting an enzymatic activity is very dependent on all the specific details of the reaction and reaction conditions. Consequently, the numeric values of enzymatic activities may vary greatly among different laboratories due to different choices of substrate and reaction conditions. Progress has been made to standardize enzyme assays by using the optimal reagent formulations defined by the International Federation of Clinical Chemistry (IFCC) reference methods.

Examples of the effect of reaction conditions and choice of units in reporting enzyme activity demonstrates that the units alone do not allow comparison of values from different laboratories.

Test analyte	Conventional units	Reference interval	SI units	Reference interval	SI conversion factor
Enzyme A (Substrate X, 37°C)	IU/L	10–50	µkat/L	0.17–0.85	0.017
Enzyme A (Substrate X, 25°C)	IU/L	3–8	µkat/L	0.05–0.14	0.017
Enzyme B (Substrate Z, 37°C)	IU/L	20–35	µkat/L	0.34–0.60	0.017
Enzyme B (Substrate J, 37°C)	IU/L	100–300	µkat/L	1.7–5.1	0.017

Important concept: Enzymatic activities reported in the same units cannot be compared if tested under different reaction conditions.

ANALYTES THAT CANNOT BE EXPRESSED IN TERMS OF MOLECULES OR MOLES

Sometimes an analyte is not a single molecule but may represent a group of heterogenous molecules with many different molecular weights. Some examples include tests for total protein, which measure all the different proteins in a sample. No single molecular weight can be used to reflect this mixture and an expression of moles per liter would be meaningless. Sometimes a molecule does not have a well-defined molecular weight and is better reported as mass rather than moles of material. Examples include proteins such as prostate-specific antigen, C-reactive protein and alpha-fetoprotein, whose molecular weights are not well established. In these cases, the SI reporting system, like the conventional system, uses a mass value to reflect the amount of material present. However, there may be differences in the reported units between the two systems, as illustrated below.

The following table shows examples of analytes reported in mass units in both conventional and SI systems.

Test analyte	Conventional units	SI units	SI conversion factor
C-reactive protein	mg/dL	mg/L	10
Alpha-fetoprotein	ng/mL	µg/L	1
Total protein	g/dL	g/L	10
Immunoglobulin M	mg/dL	mg/L	10

REVIEW QUESTIONS: SECTION 8

Answers are provided at the end of this Learning Guide.

1. Which of the following units would be used for reporting glucose on a clinical chemistry laboratory report?

    A. mg/dL

    B. ounces/L

    C. mL/L

    D. All are acceptable units

2. What would be the value of 150 mg/dL glucose reported in SI units?

    A. 1.61 mmol/L

    B. 8.25 mmol/L

    C. 0.367 mmol/L

    D. None of the above values

3. If total cholesterol is 4.0 mmol/L, what is the value in conventional units?

    A. 154 mg/dL

    B. 102 mg/dL

    C. 40 mg/dL

    D. None of the above values

4. If the enzymatic activity of LD is 40 IU/L at 25°C, what is the activity at 37°C?

    A. 40 IU/L

    B. 59 IU/L

    C. 27 IU/L

    D. Impossible to tell from information provided

APPENDICES

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APPENDIX A: GLOSSARY OF TERMS

Absorbance: The amount of light that is absorbed by analyte in a solution; absorbance is directly proportional to the concentration of analyte.

Acidosis: State of decrease of basic (alkali) compounds and an accumulation of acid compounds in the blood causing a decrease in pH.

Accuracy: Ability of a test to obtain the known target value for a sample; an accurate test exhibits minimal bias and imprecision.

Addison's disease: Chronic adrenocortical insufficiency.

Adipose: Of or relating to fat tissue in the body; tissue rich in lipids.

Alkalosis: State of excess of basic (alkali) compounds or loss of acidic compounds in the blood causing an increase in pH.

Amino acid: Organic acid that is the building block for proteins.

Analyte: Substance that is being measured (e.g., glucose, sodium, cholesterol).

Analytical measuring interval (AMI): The interval in which specimen concentrations are measured within the medical and laboratory needs for accuracy with no dilution, concentration or other pretreatment not part of the standard or routine measurement process.

Analytical phase: All procedures related to the testing of a sample for an analyte.

Antibody: An immunoglobulin protein produced by the body's immune system as the result of antigenic stimulation.

Antigen: A foreign substance that results in an immune response and antibody production.

Atomic absorption: A spectrophotometric method in which the analyte is an element (e.g., Ca), and it absorbs light at a specific wavelength. Decreases in light intensity hitting a photodetector correspond to increased analyte concentrations.

Beer's Law: The basic equation that relates analyte concentration to spectrophotometric absorbance.

Bias: The error observed for a test method; the larger the bias, the less accurate a test.

Bilirubin (icterus): Yellow discoloration of plasma caused by the breakdown of hemoglobin resulting in bilirubin accumulation.

Bilirubinemia: Presence of bilirubin in the blood.

Body fluid: Fluid in body cavities or spaces (e.g., pleural, abdominal, pericardial and synovial fluid).

Calibration: Process of using calibrators (samples with known analyte concentration) to construct a calibration curve used to quantitte analyte concentration in unknown (patient) specimens.

Cardiovascular disease (CVD): Disease of the heart and cardiac arteries due to buildup of lipid deposits or other causes of heart malfunction; a variety of analytes are used to detect and monitor CVD.

Catalyst: Substance that accelerates a chemical reaction, such as an enzyme in the body.

Cation: An ion carrying a positive charge.

Collection tubes: The various types of devices used to collect blood specimens; glass or plastic, with or without anticoagulants and/or gel separators.

GLOSSARY OF TERMS

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

Concentration: Amount of analyte measured in a sample expressed quantitatively (e.g., mg/dL, mmol/L).

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

Diabetes: Very common disease of glycemic control; blood sugar (glucose) concentrations are abnormally increased due to the inability to either produce or utilize insulin.

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

Drugs of abuse (DOAs): Illegal drugs (e.g., LSD, cocaine) or prescription drugs (e.g., amphetamines, opiates) that are used for recreational purposes.

Electrolytes: Cations (e.g., Na, K) and anions (Cl) measured in samples.

Enzyme: Protein in the body that acts as a catalyst and converts substrate to product.

Enzymatic activity: A measure of the amount of enzyme catalytic activity found in a sample; enzyme concentration is often expressed in terms of activity instead of quantitative units.

Estimated glomerular filtration rate (eGFR): An estimate of GFR using a commonly measured analyte, creatinine or cystatin C, and an equation that adjusts for various factors that influence GFR.

Extracellular: Component found outside the cell.

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

HDL (High Density Lipoprotein): A lipoprotein particle found in blood that is composed of a high proportion of protein with little triglyceride and cholesterol, and is associated with reduced risk of atherosclerosis.

Hemoglobin: Protein in red blood cells that transports oxygen from the lungs to tissues.

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

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

HIL: Hemolysis, icterus and lipemia; the most common interferents found in blood specimens.

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

Homeostasis: State of balance in the body.

Icterus (bilirubin): Yellow discoloration of plasma caused by the breakdown of hemoglobin resulting in bilirubin accumulation.

Immunoassay: Assay that relies on an antigen-antibody reaction.

Intracellular: Component found inside the cell.

Ion-selective electrode (ISE): A potentiometric device used to selectively measure individual electrolytes such as Na, K and Cl.

LDL (Low Density Lipoprotein): Lipoprotein particle found in blood composed of protein, with little triglyceride and high proportion of cholesterol, and is associated with increased risk of developing atherosclerosis.

Lipemia: Milky coloration of plasma caused by increased lipid accumulation, usually triglycerides.

Lipids: The common analytes of cholesterol and triglycerides and related compounds such as free fatty acids and lipoproteins.

Matrix: The biological fluid that is collected and used to test for an analyte (e.g., blood, urine) or the form of the biological fluid that is tested (e.g., serum, plasma).

Matrix effects: Interference effect of a sample matrix causing a false decrease or increase in a test result; common matrix interferents are hemolysis, icterus and lipemia.

Metabolites: Products of anabolism and catabolism; analytes created by synthesis in the body (e.g., glucose, cholesterol) or breakdown (e.g., creatinine, urea).

Method/methodology: The basic measurement principle or technique that is used in an analytical system to perform a test.

NADH: Nicotinamide adenine dinucleotide.

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, that leads to softening of bones.

Panel: A group of related tests ordered together.

Photometry: Measuring light intensity at various wavelengths.

Plaque: Lipid deposits in arteries causing stenosis and leading to cardiovascular disease.

Plasma: The clear, yellow fluid obtained when blood is drawn into a tube containing anticoagulant; the clotting factors have not been activated and a clot is not formed (usually a purple, green or light-blue tube).

Postanalytical phase: All procedures related to specimen handling and result reporting after the analytical (testing) phase.

Potentiometry: Measurement of electrical potential difference between two electrodes in an electrochemical cell; the methodology used by an ion-specific electrode.

Preanalytical phase: All procedures related to specimen collection and handling that precede the analytical (testing) phase

Precision: The reproducibility of a test; the ability to obtain very similar quantitative values on repeat testing of a sample.

Proteins: Large protein molecules such as albumin and immunoglobulins (IgA, IgG, IgM).

Reaction velocity: Describes the speed at which a detection measurement changes over time.

Reagent: A chemical mixture to which a sample is added to conduct a test.

Reference interval: The expected normal concentration range for an analyte in a patient population; often varies with age, gender or other partitioning factors.

GLOSSARY OF TERMS

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 upper respiratory tract infections.

Sample: The specimen after preparation for analysis (e.g., serum or plasma after centrifugation).

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

Specimen: The type of biologic fluid in which the analyte is found (e.g., blood, urine, CSF) or the form in which the fluid is tested (e.g., serum, plasma, whole blood).

Spectrophotometry: Measuring light intensity at various wavelengths.

Test: The overall process for detecting and measuring an analyte.

Therapeutic drug monitoring (TDM): Testing for common therapeutic drugs (e.g., digoxin, theophylline, valproic acid) to determine if the concentration is in the therapeutic range, below it or above it (toxic range).

Titer: The amount of antibody found in a specimen as a result of exposure to an antigen; a high titer typically occurs after an immune response and the titer decreases over time after exposure to the antigen.

Toxicology: Analysis of therapeutic drugs or drugs of abuse.

Traceability: Anchoring the calibrators of a test method to recognized reference materials and/or reference methods to ensure accuracy of results; described by a metrological traceability chain.

Urine: The aqueous waste fluid produced by the kidneys; the next most common body fluid after blood used for testing.

APPENDIX B: REFERENCES

GENERAL CLINICAL CHEMISTRY TEXTBOOKS

Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics, 5th and 7th Editions, edited by Carl A. Burtis, Edward R. Ashwood, and David E. Bruns. W. B. Saunders Company, Philadelphia, PA, 2015.

Clinical Diagnosis and Management by Laboratory Methods, 19th and 20th Editions, edited by John Bernard Henry, Frederick R. Davey, Chester J. Herman, et al. Saunders, Philadelphia, PA, 2001.

Clinical Chemistry: Theory, Analysis, Correlation, 5th Edition, 2009. Edited by Lawrence A. Kaplan, Amadeo J. Pesce and Steven Kazmierczak.

Clinical Diagnostic Technology – The Total Testing Process, 2003, Volume 1: The Preanalytical Phase. Edited by Kory M. Ward-Cook, Craig A. Lehmann, Larry E. Schoeff and Robert H. Williams.

Clinical Diagnostic Technology – The Total Testing Process, 2005, Volume 2: The Analytical Phase. Edited by Kory M. Ward-Cook, Craig A. Lehmann, Larry E. Schoeff and Robert H. Williams.

Clinical Diagnostic Technology – The Total Testing Process, 2006, Volume 3: The Postanalytical Phase. Edited by Kory M. Ward-Cook, Craig A. Lehmann, Larry E. Schoeff and Robert H. Williams.

Contemporary Practice in Clinical Chemistry, 2006. Edited by William Clarke and D. Robert Dufour.

Basic Method Validation, 3rd Edition, 2009. James O. Westgard, with contributions from Elsa F. Quam, Patricia L. Barry, Sharon S. Ehrmeyer and R. Neill Carey.

ONLINE RESOURCE FOR INTERPRETATION OF CLINICAL LABORATORY TESTS

Lab Tests Online®. U.S. site www.labtestsonline.org provides links for sites in other countries and in a number of languages.

ORGANIZATIONS PROVIDING SERVICES AND EDUCATIONAL MATERIALS

National Institutes of Standardization and Technology (NIST®): www.nist.gov

American National Standards Institute® (ANSI®): www.ansi.org

World Health Organization (WHO): www.who.int/en/

Clinical and Laboratory Standards Institute® (CLSI® — formerly NCCLS): www.clsi.org

International Federation of Clinical Chemistry (IFCC): www.ifcc.org

Institute for Reference Materials and Methods (IRMM): crm.jrc.ec.europa.eu

National Institute for Biologic Standards and Control (NIBSC): www.nibsc.org

American Diabetes Association® (ADA): www.diabetes.org

National Cholesterol Education Program (NCEP): www.nhlbi.nih.gov/health-topics/all-publications-and-resources/third-report-expert-panel-detection-evaluation-and-0

National Kidney Foundation® (NKF®): www.kidney.org/kidneydisease/ckd/

International Bureau of Weights and Measures (BIPM®): www.bipm.org

The Joint Committee for Traceability in Laboratory Medicine (JCTLM): www.bipm.org/en/committees/jc/jctlm

American Heart Association®. Highlights of the 2018 Guideline on the Management of Blood Cholesterol. 2018. healthmetrics.heart.org/highlights-of-the-2018-guideline-on-the-management-of-blood-cholesterol/

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ORGANIZATIONS PROVIDING STANDARDIZATION PROGRAMS

Cholesterol Reference Method Laboratory Network: www.cdc.gov/labstandards/crmln.html

National Glycohemoglobin Standardization Program: www.ngsp.org

IFCC HbA1c Standardization Program: www.ifcchba1c.net

ONLINE RESOURCE FOR BIOLOGIC VARIATION AND SETTING TARGET ACCURACY RANGES

Westgard QC: www.westgard.com/guest17.htm

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APPENDIX C: CORRECT RESPONSES

SECTION 1

1. A. Calcium
2. Any five of the following, e.g., Blood, Urine, CSF, Pleural, Synovial, Peritoneal, Pericardial, Saliva, Amniotic
3. C. Test samples from healthy people
4. C. 1 in 100
5. D. No additive

SECTION 2

1. B. Electrolytes
2. A. Endpoint (end-up)
3. A. Immunoturbidimetry
4. B. Between 2 and 3 nmol/L

SECTION 3

1. A. Blanking
2. A. Measurement of lipase activity
3. B. Remove substances that could be erroneously measured as analyte
4. B. Microscopy

SECTION 4

1. A. 50, 51, 52
2. B. 95, 100, 105
3. C. Both methods are showing a matrix effect for the QC material
4. C. The method's accuracy is linked to a certified method and/or material
5. B. Triglycerides

SECTION 5

1. B. Collection of blood in wrong kind of tube
2. A. Instrument not properly calibrated
3. B. Presence of interfering substances in sample
4. All

SECTION 6

1. B. Prealbumin
2. B. Ammonia
3. A. Amylase and lipase
4. D. Acetaminophen at 250 μg/mL
5. B. BNP

SECTION 7

1. C. Hemoglobin A1c
2. B. LDL
3. C. Troponin
4. B. Free T4
5. D. Iron deficiency
6. B. High blood creatinine

SECTION 8

1. B. ounces/L
2. B. 8.25 mmol/L
3. A. 154 mg/dL
4. D. Impossible to tell from information provided

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