CHAPTER e53

The Clinical Laboratory in Modern Health Care

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Modern medicine relies extensively on the clinical laboratory as a key component of health care. It is estimated that in current practice, at least 60–70% of all clinical decisions rely to some extent on a laboratory result. For many diseases, the clinical laboratory provides essential diagnostic information. As an example, histopathologic analysis provides basic information about histologic type and classification of tumors and their degree of invasion into adjacent tissues. Microbiologic testing is required to identify infectious organisms and determine antibiotic susceptibility. For many common diseases, expert groups have produced standard guidelines for diagnosis that rely on defined clinical laboratory values, e.g., blood glucose or hemoglobin A1C levels form the basis for diagnosis of diabetes mellitus; the presence of specific serum antibodies is required for diagnosis of many rheumatologic diseases; and serum levels of cardiac markers are a mainstay in diagnosis of acute coronary syndromes.

The ever-increasing number and scope of clinical laboratory tests provides the clinician with a powerful set of tools but poses the challenge of appropriate selection of clinical laboratory tests in the most judicious and cost-effective way to deliver effective patient care.

RATIONALE FOR PERFORMING CLINICAL LABORATORY TESTS

DIAGNOSIS OF DISEASE

One of the most frequent reasons for performing clinical laboratory tests is to support, confirm, or refute a diagnosis of disease that is suspected based on other sources such as the patient's history, physical examination findings, and imaging studies. The questions that need to be considered are which clinical laboratory tests could be of value in supporting, confirming, or excluding the clinical impression? What is the most efficient test-ordering strategy? If a test result is positive, will that confirm the clinical impression or even formally establish the diagnosis? If negative, does that disprove the clinical suspicion, and what further testing or approach is needed? What are the known sources of false-positive and false-negative results, and how does one recognize these?

SCREENING FOR DISEASE

Another reason for ordering clinical laboratory tests is screening for disease in asymptomatic individuals (Chap. 4). Perhaps the most common examples of this are the newborn screening programs now being used in most developed countries. Their purpose is to identify newborns with treatable conditions for which early intervention, even before clinical symptoms develop, is known to be beneficial. Screening of adults for the presence of diabetes mellitus, renal disease, prostate cancer [by testing serum prostate-specific antigen (PSA) levels], and colorectal cancer (by testing for occult blood in stool) are examples of widely used clinical laboratory screening procedures that are applied to apparently healthy individuals on the basis that early diagnosis and intervention in patients with these diseases leads to improved long-term outcomes.

Differences between screening tests and confirmatory tests

It is important to distinguish between clinical laboratory tests that can be used for screening for disease and those that offer a confirmatory result. Screening tests are generally less expensive and more widely available than are confirmatory tests, which often require more specialized equipment or testing personnel. As a general principle, screening tests are designed to identify all subjects who have the disease of interest, even if that means incorrectly labeling some healthy individuals as possibly having disease. Stated more formally, the diagnostic sensitivity of screening tests is maximized and this inevitably comes at the expense of reduced diagnostic specificity. Confirmatory testing is intended to correctly separate those individuals with disease from those who do not have disease.

As an example of these principles, when screening for hepatitis C viral (HCV) infection, a common approach is to first test for the presence of anti-HCV antibodies in a patient's serum. A positive result generally indicates either a current infection or a previous infection that the patient's immune system has successfully cleared. In the latter situation, anti-HCV antibodies may persist and be detectable for life. However, a small number of patients will have false-positive results in the serologic screening test for HCV. To resolve these uncertainties, a positive serologic screening test should be followed by confirmatory identification of hepatitis C viral RNA using molecular techniques. This confirmatory testing can provide evidence of current viral infection or identify patients who are not infected.

RISK ASSESSMENT OF FUTURE DISEASE

Another reason for clinical laboratory testing is assessing a patient's risk of developing disease in the future. A number of diseases are associated with well-established clinical laboratory-defined risk factors, which, if present, would indicate the need for more frequent monitoring for disease. The need for risk assessment is even clearer if there are also useful interventions that decrease the risk of developing disease. For example, hypercholesterolemia is a well-established risk factor for coronary artery disease that may be modified by pharmacologic intervention (Chap. 241). Many genetic mutations are known to be associated with increased risk of cancer, such as hereditary mutations in the *BRCA1* and *BRCA2* genes, which predispose to breast and/or ovarian cancer. Individuals who are known to carry these mutations require more vigilant monitoring for early signs of cancer and may even opt for prophylactic surgery in an attempt to prevent cancer (Chap. 63). Individuals with factor V Leiden are at increased risk of developing deep venous thrombosis and may benefit from prophylactic anticoagulation in the perioperative period. For example, some types of surgery, such as hip replacement, are accompanied by prolonged immobilization, which is itself an additional risk for deep venous thrombosis.

MONITORING DISEASE AND THERAPY

Many clinical laboratory tests offer useful information on the progress of disease and the response to therapy. As an example, one might consider the role of viral load measurements in patients with HIV-1 infection who are on anti-retroviral agents. According to current Centers for Disease Control (CDC) guidelines, a successful anti-retroviral response is defined by a fall in plasma HIV-1 levels of 0.5 $\log_{10}$ copies/mL, and a key goal of treatment is a reduction in the viral load to below the level of detection, which is typically in the range of 40–75 copies/mL. Other examples of the use of clinical laboratory testing for monitoring disease include measurement of tumor markers such as PSA, especially following surgical removal of tumors. In this situation, the expectation is that successful treatment of a tumor will cause a decrease in the level of the tumor

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marker. If there is a later increase in the level of the tumor marker, it suggests a recurrence of the disease. Finally, the clinical laboratory offers direct monitoring of levels of some therapeutic agents such as drugs. This monitoring is important if a drug has a defined therapeutic concentration range, above which it is toxic and below which it is ineffective. Monitoring of drug levels in this situation facilitates optimum dosing and avoidance of toxicity.

CRITICAL VALUES

Clinical laboratories are required to establish a list of "critical values." These are values of test results that indicate an immediate risk of jeopardy to the health or life of the patient and therefore require urgent communication to the patient's physician so that appropriate medical intervention may be accomplished. Critical values are reported regardless of whether the test was ordered as a "stat" or routine test. The critical values themselves are generally created by the clinical laboratory medical director in conjunction with the medical staff. A representative list of critical values is shown in Table e53-1.

"STAT" ORDERS

Tests that are ordered as "stat" receive priority in the clinical laboratory's testing queue, which means that other patient specimens may be delayed while a "stat" specimen is analyzed. Ordering a test "stat" should be reserved for situations in which a result is needed for urgent medical care; this is a judgment that must be made by the ordering physician. "Stat" testing should not be used merely for convenience of either the patient or the health care provider.

SENSITIVITY AND SPECIFICITY IN THE CLINICAL LABORATORY

The commonly used metrics of a clinical laboratory test are the diagnostic sensitivity, specificity, and positive and negative predictive values. These concepts are discussed in Chap. 3. In the clinical laboratory, the terms *sensitivity* and *specificity* have alternative meanings that are applied to tests, and the different uses of these terms may cause confusion. Analytic sensitivity can refer to the lowest detectable concentration of analyte that can be measured with some defined certainty, or to the rate of change of signal intensity as analyte concentration changes. As an example, newer "generations" of laboratory assays frequently have improved sensitivity over earlier generations, meaning that they can detect lower concentrations of the analyte, which is often of value in disease diagnosis. Analytic specificity refers to the extent to which other substances in the test system interfere with measurement of the analyte of interest. This concept is frequently applied to immunoassays, in which a detection antibody may also bind with compounds that have a similar structure, e.g., immunoassays for drugs may show cross-reactivity with drug metabolites, and immunoassays for steroids may show cross-reactivity with other steroids of similar structure. Certain chemical assays are also subject to nonspecificity. For example, a common chemical method used to measure creatinine, the Jaffe reaction, shows positive interference from a number of other compounds including glucose, certain ketones, and cephalosporin antibiotics. Elevated concentrations of bilirubin, free hemoglobin, and the presence of turbidity in plasma or serum specimens may also interfere with some assays. The clinical laboratory should be able to provide advice about the presence or magnitude of these effects in assays that it performs.

CLINICAL LABORATORY DIAGNOSTIC PRINCIPLES

Clinical laboratory diagnosis, like all diagnosis, is based on disease-related changes from normality.

1. Tissue injury or necrosis allows leakage of intracellular components into the circulation leading to detectable rises in blood levels of these components. Many intracellular molecules are

TABLE e53-1 Selected Examples of Laboratory Critical Values

	Less than	Greater than
CHEMISTRY		
Ammonia		>100 µmol/L
Calcium, ionized,	<3 mg/dL	>7 mg/dL
Calcium, total	<6 mg/dL	>14 mg/dL
Carboxyhemoglobin		>10%
Creatine kinase, total		>1000 U/L
CO_2, total	<11 mol/L	>45 mmol/L
Digoxin		>2.5 µg/L
Glucose	<40 mg/dL	>500 mg/dL
Glucose—CSF	<40 mg/dL	
Ketone		>1.5 mmol/L
Lithium		> 2.0 mmol/L
Magnesium		>7 mg/dL
Methemoglobin		>35%
Osmolality	<250 mmol/L	>340 mmol/ L
Phosphorus	<1 mg/dL	
pH	<7.1	>7.6
P_{CO_2}	<20 mm Hg	>75 mm Hg
P_{O_2}, arterial	<40 mm Hg	
P_{O_2}, capillary	<30 mm Hg	
Bicarbonate	<11 mol/L	>45 mol/L
Potassium	<2.7 mmol/L	>6 mmol/L
Salicylate		>30 mg/L
Sodium	<120 mmol/L	>160 mmol/L
Troponin		>0.120 µg/L
HEMATOLOGY		
INR		>6.0
PTT		>105 s
Hemoglobin	<7 g/dL	
WBCs	$<1 \times 10^9$/L	$>50 \times 10^9$/L
Platelets	$<50 \times 10^9$/L	$>1000 \times 10^9$/L
MICROBIOLOGY (any positive result below is critical)		
Acid-fast culture or smear		
Blood culture		
CSF Gram stain/culture		
CSF cryptococcal antigen		
Malarial smear		

Abbreviations: CO_2, carbon dioxide; CSF, cerebrospinal fluid; INR, international normalized ratio; P_{CO_2}, partial pressure of carbon dioxide; P_{O_2}, partial pressure of oxygen; PTT, partial thromboplastin time; WBCs, white blood cells.

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common across tissue types, and are therefore not indicative of injury to a specific tissue. Other constituents are selectively expressed in relatively high concentrations, or even uniquely present, in certain tissues. Therefore, their presence in the blood is evidence of injury to that tissue. This principle forms the rationale for measurement of blood levels of, for example, liver enzymes in evaluating liver disease (Chap. 302), cardiac troponins in acute coronary syndromes (Chap. 245), and myoglobulin in muscle injury. The extent of the rise in blood levels of these markers generally correlates with the extent of tissue damage, although there are exceptions; for example, liver enzyme levels may fall in end-stage liver disease.

2. An increase in blood levels of some analytes indicates failure of normal excretory processes. This principle is demonstrated by, for example, elevations in conjugated bilirubin that accompany obstruction of the biliary system, by elevations in ammonia that are seen in advanced or metabolic liver disease, by rises in creatinine and potassium levels in renal failure, and by increases in P_{CO_2} in some pulmonary diseases.
3. Increases in blood concentration of tissue-specific markers may result from expansion of the total volume of that tissue. This principle forms the basis for the use of measurement of levels of many tumor markers such as PSA (prostate cancer), CA-125 (ovarian cancer), CEA (colon cancer), and CA-19-9 (pancreatic cancer). In practice, the usefulness of these markers varies according to the degree to which they are produced by a tumor and by the tumor size. Small colon cancers, for example, may not produce a significant rise in CEA levels whereas small prostate cancers often produce detectable rises in PSA concentrations.
4. Disease processes often manifest characteristic patterns of coincident changes in levels of several analytes. These patterns of change can be understood by consideration of the underlying pathophysiology. For example, acute intravascular hemolysis is characterized by a fall in levels of hemoglobin and haptoglobin and by a rise in unconjugated bilirubin. In endocrine diseases, there are often changes in concentrations of several hormones because of disturbance of feedback loops. Primary hyperthyroidism, as an example, is characterized by increases in thyroxine (T_4), and by suppression of thyroid-stimulating hormone (TSH). In diabetic ketoacidosis caused by insulin deficiency, there are concomitant elevations of plasma glucose, ketones, and frequently, potassium. In response to the metabolic acidosis, levels of bicarbonate are typically reduced.
5. Genetic changes underlie many diseases, both inherited and acquired. In the era of molecular medicine, there is increasing recognition of the contribution of hereditary factors to many common diseases. Often, the epidemiology of common diseases such as hypertension is characterized by a minority of families that have mutations in recognized genes, whereas in the larger population, the genetic basis of the same disease phenotype is unclear. The search for the genetic factors that contribute to many common diseases remains a topic of intense research interest. It is now clear that essentially all tumors have genetic abnormalities. Although there is an inherited predisposition in some families, most of these genetic changes are acquired. Identification of the genetic abnormalities in cancer offers new tools for clinical laboratory diagnosis and classification of tumors in ways that surpass traditional histopathology and also offers insights into cellular processes that may be targets for treatment.
6. Clinical laboratory results should always be interpreted in the context of the patient's history and physical examination and any other relevant information (e.g., imaging studies); the clinician should avoid treating laboratory results rather than the patient.
7. Recommended clinical laboratory tests change with time. As new markers of disease emerge, they may replace older markers, for example, measurement of serum creatine kinase (CK) levels was introduced for diagnosis of acute myocardial infarction in the 1980s. Use of the cardiac-specific isoenzyme CK-MB later became widespread in clinical practice. Today, cardiac troponins are replacing CK (or CK-MB) measurements in recommended guidelines. Many other assays have fallen out of use as better assays have become available. Measurement of urine 17-ketosteroids (arising from androgens) and of urine 17-hydroxycorticosteroids (arising from glucocorticoids) has been supplanted by immunoassay of specific steroid hormones. Today, many steroid hormones are measured by mass spectrometry, which often provides improved analytic specificity over immunoassays. As new tests are introduced, it is essential that they be evaluated critically before adoption for clinical use. At a minimum, consideration needs to be given to questions of clinical validation, specimen stability, diagnostic sensitivity and specificity, positive and negative predictive values, analytic accuracy and precision, and costs.

REFERENCE RANGES

When interpreting clinical laboratory results, comparison is usually made to a reference range (sometimes called a normal range) that defines the values seen in health or considered to be desirable for health. Several common methods are used to describe reference ranges in the clinical laboratory.

1. For many quantitative clinical laboratory tests, the range of observed values seen in a healthy population shows an approximately Gaussian distribution. The factors that contribute to this range include the inter- and intraindividual variation in the concentration of the analyte, and the analytic imprecision. When there is an approximately Gaussian distribution of values in the population, the reference range is commonly defined as being the central 95% of the range of distribution of those values. Using this method, 2.5% of the population will have a measured value that is below the reference range for the analyte, and 2.5% will have a value that is above the reference range. The fact that 5% of healthy individuals will have a test value that is outside the reference range has important implications when ordering multiple tests. If N independent tests are performed on a specimen, then the probability of at least one result being outside the reference range is $(1-0.95^N)$. The greater the number of tests that are ordered, even on a healthy individual, the greater is the likelihood of finding an abnormal result (Fig. e53-1). If 20

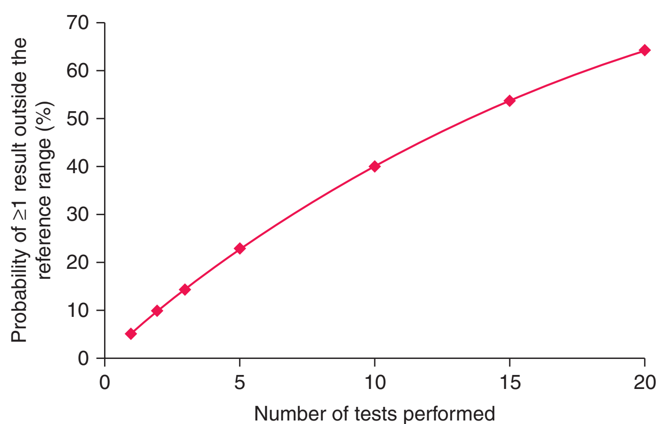

Figure e53-1 Probability of at least one laboratory result being abnormal in a healthy individual as an increasing number of independent tests are performed. The reference range is the central 95% of values measured in a healthy population.

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TABLE e53-2 National Cholesterol Education Program Adult Treatment Panel III Guidelines for Cholesterol	
LDL Cholesterol	**(mg/dL)**
Optimal	<100
Near Optimal/Above Optimal	100–129
Borderline High	130–159
High	160–189
Very High	≥190
Total Cholesterol	**(mg/dL)**
Desirable	<200
Borderline High	200–239
High	≥240
HDL Cholesterol	**(mg/dL)**
Low	<40
High	≥60

independent tests are performed on a healthy subject, the probability of having at least one abnormal result is almost two-thirds.

In some settings, a narrower range of values is considered to be abnormal. For example, current American Heart Association guidelines recommend using a serum level of cardiac troponins that is greater than the 99th percentile of values found in a healthy population as evidence of acute myocardial infarction.

2. An alternative approach to using population means and standard deviations is to define a range of analyte values that is judged to be consistent with health based on expert consensus opinion. These ranges are often referred to as *decision limits*. Examples of reference ranges established this way include those for total, high-, and low-density cholesterol (Table e53-2). Such ranges may deviate considerably from those that would be established if the analyte concentrations of the population mean ± 2 standard deviations were used as a basis for establishing the reference range. For example, the "desirable" total cholesterol value according to the National Cholesterol Education Program is under 200 mg/dL. This value is actually very close to the mean concentration among U.S. adults; in fact, almost one-half of U.S. adults have a total cholesterol concentration that is above the "desirable" range. If the central 95% of cholesterol concentrations in the population were taken as the reference range, the upper end of that range would be approximately 240 mg/dL, well beyond what is considered desirable.

Reference ranges may vary with age, gender, ethnic background, and physiologic state (e.g., pregnancy, high-altitude adaptation). Some examples of these are shown in the Appendix. The existence of different reference ranges poses challenges for interpretation of results. In particular, creatinine stands out as an analyte for which conventional reference ranges are not always easy to apply in clinical practice. Plasma levels of creatinine vary with age, gender, and ethnic group. This fact makes it difficult in practice to use a simple reference range for this analyte when attempting to gauge a patient's renal function. A large decrease in glomerular filtration rate (GFR) is associated with slight increases in plasma creatinine within the typical reference range provided by many laboratories (Fig. e53-2). A 60-year-old white woman with a serum creatinine of 1.00 mg/dL, which is well within the typical reference range, has an estimated GFR of only 57 mL/min per 1.73m^2, whereas the same creatinine concentration in a 20-year-old African-American male would be consistent with normal renal function. To better estimate the GFR, which is widely considered to be the most useful index of overall renal function, it has become customary to use equations that incorporate plasma creatinine with other parameters. The most widely used of these equations in current practice is the "4-parameter" Modification of Diet in Renal Disease (MDRD) equation that incorporates plasma creatinine, age, gender, and ethnic group (African American or not African American). Recommended clinical laboratory practice is to report the estimated GFR (eGFR) with

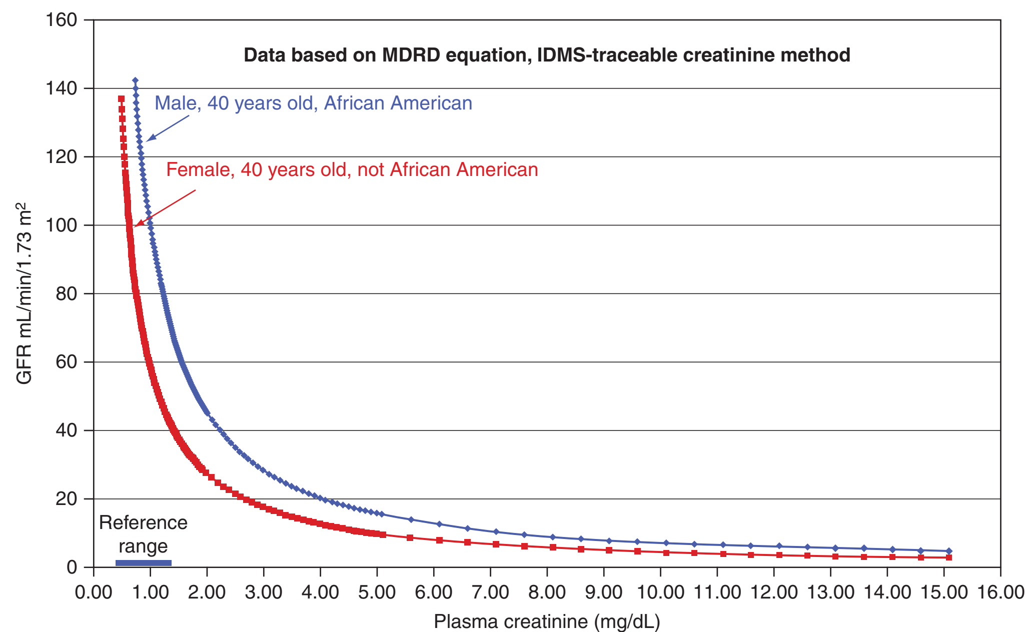

Figure e53-2 Relationship between plasma creatinine and estimated glomerular filtration rate (eGFR) using the 4-parameter Modification of Diet in Renal Disease (MDRD) equation. IDMS, isotope dilution mass spectrometry.

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all creatinine measurements in adults. This provides more useful information than would a creatinine reference range alone.

SOURCES OF ERROR IN CLINICAL LABORATORY TESTING

Errors can arise at all stages of the testing process from specimen collection to result interpretation. An error arising at any stage may adversely affect patient care. In clinical laboratory practice, it is customary to divide the testing process into three phases: preanalytic, analytic, and postanalytic. Examples of each type of error are shown in Table e53-3. The most frequent error in the testing process is specimen mislabeling, which involves a specimen from one patient being placed in a container that is labeled with another patient's name or identifiers. Specimen mislabeling errors may result in very serious consequences for a patient. For example, if a mislabeled specimen results in erroneous typing of a patient's blood group followed by transfusion of a mismatched unit of blood, the outcome may be fatal. A mislabeled biopsy specimen may lead to an erroneous diagnosis and inappropriate therapy for a patient or, alternatively, failure to make a diagnosis and institute appropriate therapy.

In addition to errors, many preanalytic factors can influence clinical laboratory results. Posture (i.e., recumbent versus upright), exercise, diet, recently ingested food, and use of prescribed or recreational drugs including tobacco, alcohol, caffeine, and herbal supplements can influence a variety of analyte concentrations. After blood has been collected certain analytes undergo changes in their concentration during storage or transportation. Glucose levels fall as a result of red cell metabolism. Ammonia levels rise as a result of protein breakdown. Increasing permeability and breakdown of red cell membranes leads to increases in plasma potassium and free hemoglobin levels. Bacterial contamination can lead to overgrowth of specimens. To minimize these precollection alterations, specimens should be processed or transported to the clinical laboratory as soon as possible after collection. The list of known preanalytic variables and their effects are extensive, and the reader is referred to the compendium on this subject by Young (see "Further Readings").

TABLE e53-3 Examples of Preanalytic, Analytic, and Postanalytic Errors During the Laboratory Testing Process

Preanalytic Sources of Error
Test selection
Inappropriate test for the clinical need
Lack of clinical usefulness regardless of possible results
Test order misunderstood or not communicated
Specimen collection
Incorrect time of collection
Patient not prepared for collection (e.g., not fasting)
Incorrect specimen type (e.g., wrong anticoagulant, wrong tissue fixative)
Use of incorrect specimen container
Insufficient specimen collected
Contamination of specimen by IV fluids, drugs, or bacteria
Specimen mislabeled or unlabeled
Important clinical information not provided
Delays in transportation to the lab leading to alterations in specimen constituents
Analytic Sources of Error
Incorrect storage conditions prior to analysis
Specimen misidentification in the laboratory
Wrong test performed
Assay interferences
Assay failure (e.g., assay out of control)
Postanalytic Sources of Error
Delay in communication of assay results
Results not communicated to correct person
Incorrect result communicated
Misinterpretation of result

POINT-OF-CARE TESTING

The great majority of tests continue to be performed in dedicated clinical laboratory facilities, but for several decades there has been a trend toward performing point-of-care testing. This has been made possible by the development of portable analytic devices, which include single-purpose instruments such as glucometers and oxygen saturation monitors, and multifunction instruments that can perform a wider variety of analyses, particularly in chemistry and hematology, but also in some areas of microbiology. The use of these devices is driven largely by the convenience offered by faster result availability. In some settings such as rural areas and developing countries there may not be an easily accessible clinical laboratory, and in such settings a point-of-care device may be the best or only option for testing. However, the per-specimen cost of point-of-care testing both in terms of reagents and supplies and of personnel is often greater than that offered by centralized testing. There are also concerns about the adequacy of personnel training to perform point-of-care testing, the quality of the results, and the incorporation of results into the medical record.

HOME TESTING BY PATIENTS

One of the largest markets for point-of-care testing is home testing by patients, which has long been an important element in the management of patients with diabetes who monitor their own blood glucose levels. Over-the-counter kits for home pregnancy testing have been available for decades. More recently, kits have become available for home testing of the international normalized ratio (INR) or prothrombin time by patients on oral anticoagulants. Kits are also available for cholesterol monitoring, fecal occult blood detection, and hemoglobin measurements. This is an area where there is often little information on the quality of test performance, the accuracy of the results, or the correct interpretation.

ISSUES SPECIFIC TO GENETIC TESTING

The principles of genetic medicine in clinical practice are discussed in Chaps. 61–63. Here we will concentrate on issues related to clinical laboratory testing for genetic disease.

The distinction between genetic testing for inherited versus acquired disorders affects the type of tissue that should be obtained for analysis. In inherited disorders, all nucleated cells are expected to carry the inherited mutation, and thus white blood cells or buccal cells (obtained by scraping the inside of the cheek) are convenient sources of DNA for clinical laboratory testing. For prenatal testing of the fetus, chorionic villi or amniocytes are commonly used. When testing for acquired genetic disorders such as in tumors, the tissue of interest that contains a suspected mutation must be sampled to obtain suitable genetic material for testing. It is often useful to compare the tumor DNA with the patient's normal DNA

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to identify acquired mutations, for example in testing for microsatellite instability in colorectal cancer (Chap. 83).

INFORMED CONSENT FOR GENETIC TESTING

Although it is assumed that all clinical laboratory testing is performed with the consent of the patient or, in the case of minors, the parents, there may be regulatory requirements to obtain formal written consent for genetic testing. Such regulations vary between jurisdictions, and the practicing clinician should be aware of any local regulations. In some jurisdictions there are regulations on the storage and use of genetic information and on the duration of time for which genetic specimens may be stored.

For some late-onset genetic diseases, such as Huntington's disease (Chap. 372), genetic testing allows for a prediction of whether a patient will develop the disease in the future. The degree of certainty that is possible from this testing surpasses that associated with identification of typical disease risk factors such as hyperlipidemia as a risk for future myocardial infarction. It is important when deciding to undertake predictive genetic testing that the patient considers the broad implications of a positive or negative test result, is made aware of any support and counseling that is available, and understands the implications of a result for other family members. In dealing with these issues, genetic counselors play an important role (Chap. 63). Their expertise includes the ability to explain genetic disorders at an understandable level to patients and their families, arrange for support services, and provide genetic risk assessments to members of families with genetic disorders.

When testing for genetic disorders, the clinical laboratory will use different analytic approaches according to the disease of interest. Some disorders, such as sickle cell anemia, are caused by single-point mutations. Testing for these disorders involves merely testing for one or a few mutations in a single gene. Other disorders (e.g., hyperphenylalaninemias) may be caused by numerous mutations in a single gene, while others (e.g., hereditary breast cancer) may be caused by mutations in many genes. The number of possible mutations and genes that underlie a clinical phenotype affects the cost and time required to perform clinical laboratory testing, and the likelihood of finding a disease-causing mutation.

If a disease phenotype can be caused by many mutations, a clinical laboratory result that is negative should be interpreted with care. As an example, it is common to screen healthy pregnant women (and their partners) for mutations in the *CFTR* gene, which is mutated in patients with cystic fibrosis (CF). The goal of this screening is to identify women who are carriers of a *CFTR* mutation and therefore at increased risk of having a baby with CF. Because CF is an autosomal recessive disorder, a fetus has a 1:4 chance of being affected if both parents are carriers of disease-causing *CFTR* mutations. The screening test approach that is commonly used to identify mutations in carriers detects 80–85% of all known disease-causing *CFTR* mutations in whites and up to 97% of mutations among Ashkenazi Jewish people. A negative screening result therefore does not completely eliminate the possibility that a woman (or her partner) actually has a mutation. What can be inferred from a negative test result is that the risk of having a CF-affected baby has decreased significantly to an extent that depends on her ethnic group and the mutations that were examined. The clinical laboratory should calculate and report her new risk of being a carrier if the screening result is negative.

LIMITATIONS TO MOLECULAR GENETIC TESTING

Genetic testing has limitations that are often unique to this field. Results may be inconclusive. For example, a search for mutations in a gene that is suspected of causing a disease may fail to reveal any known disease-causing mutations. A mutation may be discovered that is of unknown clinical significance. In this situation, consideration of any change in the amino acid sequence of the protein may suggest a biologic effect, e.g., replacement of a charged amino acid by one of the opposite charge or by a neutral amino acid, or replacement of an amino acid by one of a different size, or replacement of an amino acid that is conserved across multiple species. Further information may be obtained by determining whether the mutation is found in healthy individuals. Even with all of these considerations, it is not uncommon that the biologic significance of an identified mutation remains uncertain, and further research may be needed to assess its significance.

It is also important to understand the limitations of the clinical laboratory approach used to detect mutations. Large-scale sequencing of DNA remains at this time an impractical undertaking for analyzing many genes for both technical and financial reasons, although there are a few genes for which extensive sequence analysis has become the standard of care, for example, *BRCA1* and *BRCA2* in assessing the risk of breast and ovarian cancer in individuals with a strong family history. As sequencing technologies improve and become less expensive, it can be expected that these will be more commonly used for both identifying mutations in patients with genetic disorders and in screening asymptomatic individuals at risk of genetic disease.

Another unique aspect of genetic testing is the concern that genetic information about individuals may be used to discriminate against them by employers or by insurance companies. In the United States, the Genetic Information Nondiscrimination Act of 2008 (GINA) prohibits the use of genetic information by employers in making decisions related to employment, and by health insurance companies in issuing insurance policies or setting premium rates based on knowledge of the applicant's genetic status. GINA does not cover disability insurance, long-term care insurance, or life insurance policies.

Although the focus of public attention has been most closely directed to DNA testing, it should be pointed out that other clinical laboratory investigations, not usually thought of as being genetic, may provide important genetic information about the person being tested. For example, serum protein electrophoresis may reveal α-1 antitrypsin deficiency. Measurement of hemoglobin A1C, commonly used for following diabetes control, may, depending on the clinical laboratory technology used, reveal a hemoglobin variant such as HbS (sickle cell). Measurement of cholesterol and triglyceride levels may reveal any one of a number of hereditary disorders. All of these constitute types of genetic information.

REGULATION OF THE CLINICAL LABORATORY

In the United States, all clinical laboratory testing performed for clinical purposes (but not for research purposes) is regulated by the federal Clinical Laboratory Improvement Amendments Act of 1988 (CLIA). Home monitoring by patients who are testing their own specimens is not covered by CLIA. The statute and the regulations, which are administered by the Centers for Medicare and Medicaid Services (CMS), apply to all laboratories whether they are located in a physician's office, a large hospital, or a reference laboratory; and all laboratories are required to hold a valid CLIA certificate that is appropriate for the complexity level of testing that is performed. The Food and Drug Administration is responsible for assigning the complexity level of commercial tests. The lowest category of complexity is the "waived" category. In order of increasing complexity are the categories of "provider-performed microscopy," "moderate complexity," and "high complexity" testing. The clinical laboratory CLIA certificate must reflect the highest complexity level of testing that is performed. The category of provider-performed microscopy is used

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to cover tests such as potassium hydroxide (KOH) preparations on skin scrapings examined for fungi, fern tests, and sperm motility tests. It does not apply to histopathology that falls into the high complexity category. It is important to note that even if a clinical laboratory is performing only testing in the "waived" category, it must still hold a valid CLIA certificate. Laboratories that hold certificates for nonwaived tests are required to participate in proficiency testing and are regularly inspected to monitor their performance.

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