2 INTRODUCTION TO COMMON LABORATORY ASSAYS AND TECHNOLOGY

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OBJECTIVES

After completing this chapter, the reader should be able to

- Describe the current and developing roles of laboratory testing in accurately diagnosing diseases
- Describe the basic elements of photometry and the major components of a spectrophotometer
- Explain the principles of turbidimetry and nephelometry as applied to laboratory testing
- Describe the analytic techniques of electrochemistry based on potentiometry, coulometry, voltammetry, and conductometry
- Describe the major electrophoresis techniques and their applications
- Describe the major analytic techniques of chromatography and compare gas- and high-performance liquid chromatography with respect to equipment and methodology
- Learn the basic principles of immunoassays; compare and contrast the underlying principles, methods, and tests performed involving radioimmunoassay, enzyme-linked immunosorbent assay, enzyme-multiplied immunoassay, fluorescent polarization immunoassay, and agglutination enzyme-linked immunoassay tests
- Describe the basic components of a mass spectrometry system

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THE CHANGING ROLE OF THE LABORATORY IN THE DIAGNOSIS OF DISEASE

Traditionally, the physician bases a clinical diagnosis and patient management protocol on the patient's family and medical history, clinical signs and symptoms, and data derived from laboratory and imaging tests. An accurate history and physical examination of the patient are still considered among the most informative procedures in establishing accurate diagnoses of disease, with clinical laboratory test results playing important roles in confirming and ruling out certain diseases.

Pharmaceutical companies have developed drugs based on these collective empiric observations and known disease mechanisms. Some common examples include medications for high cholesterol, which modify the absorption, metabolism, and generation of cholesterol. Agents have been developed that are aimed at improving insulin release from the pancreas and sensitivity of the muscle and fat tissues to insulin action. Antibiotics are based on the observation that microbes produce substances, which inhibit other species. Hypotensive medications that lower blood pressure have typically been designed to act on physiologic pathways involved in hypertension (such as renal salt and water absorption, vascular contractility, and cardiac output). This has often been a reactive approach with appropriate treatments and therapy starting after the signs and symptoms appear.

The past 30 years has seen remarkable progress in the role of the laboratory in personalizing medicine, a consequence of the advances in human and medical genetics. These advances have enabled a more detailed understanding of the impact of genetics in disease and have led to new disciplines: genomics, epigenetics, and proteomics. It is anticipated that discoveries in these areas will change the practice of traditional clinical medicine into a more personalized medicine approach and will also impact pharmaceutical development.

Many of the traditional laboratory procedures and tests that are described in the following parts of this chapter will create the framework upon which these potential advances will be based—researchers are simplifying them, improving throughput, and running in real time. As these tests are automated, they will take their place alongside current testing procedures. In the United States alone, each year clinicians order laboratory tests costing billions of dollars. Although most laboratory testing is not performed by these clinicians themselves, it is imperative that they have an introduction to, and a basic understanding of, the more common and newer methods and techniques used to generate this clinical data. This understanding is essential for the proper ordering of tests and, most importantly, the correct interpretation of test results. This chapter provides an introduction to these methods and techniques.

Clinical laboratory testing represents a vast array of diverse procedures ranging from microscopic examination of tissue specimens (histopathology), to measurement of cellular components, to the amplification and detection of genetic material, such as the detection of a gene mutation or fusion for malignancies or the identification of antimicrobial resistance genes in bacteria. A consideration of all of the diverse methodologies used in these procedures is beyond the scope of this chapter,

OBJECTIVES

- Understand the basic principles of the commonly used cytometry systems
- Describe the impact of genomics, epigenetics, and proteomics on the personalization of medical practice and the newer roles that laboratory tests will play in the future
- Describe the basic principles of molecular diagnostics
- Describe the basic techniques of the polymerase chain reaction

but all share some of the common characteristics of automation and mechanization. The two often intertwine; automation commonly involves the mechanization of basic manual laboratory techniques or procedures such as those described throughout this chapter. The common goals of total laboratory automation (TLA) result in increased efficiency and throughput leading to decreased turnaround times, reduced errors, and the ability to integrate various quality assurance and improvement processes in the laboratory.

AUTOMATION IN THE HOSPITAL AND CLINICAL LABORATORY

This trend toward automation in the hospital and clinical laboratory is, in part, motivated by the drive toward higher productivity and cost efficiency.[1] Another key driver clinical laboratories face is the federal government. According to a report issued from the U.S. Department of Health and Human Services, the Office of the Inspector General (OIG) stated that Medicare could have saved $910 billion (38%) on laboratory test reimbursement if they lowered the reimbursement rate for the top 20 laboratory tests.[2] A final conclusion from this report was the OIG should consider reintroducing competitive bidding and adjusting the reimbursement rate for these laboratory tests. Clinical laboratories, like many other departments in hospitals and other healthcare facilities, are facing the pressure of providing more services, while maintaining high quality standards with a reduced revenue stream. In its most comprehensive sense, TLA encompasses all procedures from receipt of the specimen to the reporting of results. System designs and functionality can vary. They can involve consolidated analyzers, individual or integrated, and automated devices that address specific tasks, coupled to specimen processing and transportation systems, as well as process control software (i.e., middleware) that automates each stage of the system. One plausible vision of the future is that the centralized hospital and clinical laboratory will consist mainly of automated laboratory systems capable of performing high volume and esoteric testing operated by skilled medical laboratory scientists.[3,4]

Laboratory automation involves a variety of steps and generally begins with processes that are manual in nature: obtaining the specimen, patient identification, transportation, and any preanalytic specimen processing. Once in the laboratory a quality control (QC) process begins with a check of the preordered specimen to ensure that specimens have correct identification labels and bar codes, the correct tube was used for the blood test ordered, and appropriate quality and adequate quantity of material is provided for the testing requested. The TLA systems are currently capable of performing only some of the above preanalytic checks. Determining whether, for example, a specimen is grossly hemolyzed, icteric, or lipemic usually requires examination by a laboratory scientist.

In many divisions of the centralized laboratory, three major areas (e.g., chemistry analyzers, hematology analyzers, and automated microbial identification systems) generate information in almost completely automated ways. Using the example of a chemistry analyzer, introduction of a specimen begins with aspiration of the sample into a continuous-flow system. Each specimen passes through the same continuous stream and is subjected to the same various analytical reactions. In some systems, the use of repeated flushing and washing steps of probes within the systems prevents carryover between specimens, while other systems use discrete specimen sampling through the use of disposable pipet tips. Many results generated by automated chemistry analyzers rely on reactions based on principles of photometry, which will be discussed later in this chapter. In addition to the more commonly requested serum chemistry levels, enzymes, therapeutic drugs, hormones, and other analytes can also be measured using these techniques.

All modern automated analyzers rely on computers and sophisticated software to perform these sample processing functions. Calculations (statistics on patient or control values), monitoring (linearity and QC), and display (acquisition and collation of patient results and warning messages and delta checks) functions are routinely performed by these instruments once the specimen has been processed. Automation does not end at this stage. Many centralized laboratories have electronic interfaces that link separate analyzers to the laboratory information system (LIS). In turn, the LIS is interfaced with the hospital electronic medical record system. This interface allows for vital two-way connectivity between the two systems. Laboratory orders are automatically sent to the LIS. This prevents errors when a manual requisition system is utilized. Also, laboratory diagnostic information can be immediately uploaded into the patient chart for review by the clinician once results are verified manually by a medical laboratory scientist or through the use of automated rule systems developed by the laboratory. Then, based on the results generated, some laboratories have created electronic rules that can automatically order repeat and reflex testing, track samples and results through the system, and manage the storage and, when necessary, the retrieval of specimens.

Standardization within the laboratory automation arena is an essential means of assuring QC and quality assurance