CONTENTS

PART V Molecular Diagnostics

1

Clinical Chemistry and Molecular Diagnostics

*Nader Rifai, Andrea Rita Horvath, Carl T. Wittwer**

OBJECTIVES

1. Define the following terms:
   - Ethics
   - Laboratory medicine
   - Molecular diagnostics
2. List and explain the reasons for performing a laboratory test.
3. Describe the field of laboratory medicine, including subdisciplines, information handling, and ethical issues.
4. Describe the role of the clinical chemist.
5. Describe the possible career paths for the clinical chemist.
6. State the applications of molecular diagnostics in laboratory medicine.
7. List and explain five ethical issues that confront laboratorians; describe the critical importance of maintaining confidentiality in the laboratory.
8. Evaluate a possible confidentiality or conflict of interest issue and determine whether it is an ethics violation.
9. State the roles of authors, editors, reviewers, and publishers in providing high quality scientific publications.

KEY WORDS AND DEFINITIONS

Ethics Rules or standards governing the conduct of an individual or the members of a profession.

Laboratory medicine A component of laboratory science that is involved in the selection, provision, and interpretation of diagnostic testing of individual specimens.

Laboratory testing A process conducted in a clinical laboratory to rule in or rule out a diagnosis, to select and monitor disease treatment, to provide a prognosis, to screen for a disease, or to determine the severity of and monitor a physiological disturbance.

Molecular diagnostics Use of molecular biology techniques to predict, prevent, diagnose, and monitor disease, including the selection and optimization of therapies.

The disciplines of *clinical chemistry* and *molecular diagnostics* elicit different images. For clinical chemistry, one thinks of pH measurements or large chemistry analyzers, whereas molecular diagnostics conjures up the human genome project, companion diagnostics, and personalized and precision medicine. Although clinical chemistry is at the core of laboratory medicine, molecular diagnostics is a more recent but explosive upstart. Clinical chemistry excels in random access testing, but molecular diagnostics has evolved massively parallel methods. On the surface, these disciplines appear clearly different.

However, consider the meaning behind the words that compose "clinical chemistry" and "molecular diagnostics." Chemistry by its very nature is molecular, and the study of molecules is chemistry. There is no difference here. Perhaps the "molecular" in "molecular diagnostics" suggests complex polymers with meaningful sequence, excluding simpler chemicals. DNA and RNA sequences largely define life, and powerful technologies for nucleic acids now eclipse those for other complex polymers such as proteins and carbohydrates. In common parlance, molecular diagnostics is dominated by nucleic acids. The words "clinical" and "diagnostics" are also similar, connecting both fields to human disease. "Clinical" is more generic than "diagnostics," but again in common use, "molecular diagnostics" includes not only diagnostics but prognosis and genetic predisposition as well. In each

* The authors gratefully acknowledge the contributions by David E. Bruns, François A. Rousseau, and Carl A. Burtis on which portions of this chapter are based.

two-word combination, the sum is greater than its parts, with combined meanings evolving to fit needs and interest. We believe that molecular diagnostics is best viewed as a subset of clinical chemistry.

According to the definition of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), "Clinical Chemistry is the largest subdiscipline of Laboratory Medicine which is a multidisciplinary medical and scientific specialty with several interacting subdisciplines, such as hematology, immunology, clinical biochemistry, and others. Through these activities clinical chemists influence the practice of medicine for the benefit of the public."

Clinical laboratories provide in vitro testing of chemical, biochemical, and genetic markers in various fluids or tissues of the human body to screen for a disease, confirm or exclude a diagnosis, help to select or monitor a treatment, or assess prognosis. Laboratory testing impacts health care delivery to virtually every patient.

LOOKING BACK

The examination of body fluids for the diagnosis of disease is certainly not a modern concept. The Greeks noticed before 400 BC that ants are attracted to "sweet urine." However, laboratory testing was not always appreciated by clinicians; the famous Dublin physician Robert James Graves (1796–1853) once remarked, "Few and scanty, indeed, are the rays of light which chemistry has flung on the vital mysteries," and the pioneer Max Josef von Pettenkofer (1818–1901) stated that clinicians use their chemistry laboratory services only when needed for "luxurious embellishment for a clinical lecture." Such views have changed throughout the years, and laboratory testing has proven to be a useful tool to clinicians who have grown to depend and rely on laboratory testing in the routine management of their patients Box 1.1.

Although it may be difficult to pinpoint the exact date when the concept of the clinical laboratory was born, all indications point to the mid-19th century. One such indication is an article titled "Hospital Construction" by Francis H. Brown that was published in the *Boston Medical and Surgical Journal*, the precursor of the *New England Journal of Medicine*, in 1861. Dr. Brown stated, "[Every hospital should have] a small room at the end of the ward to serve as a general laboratory … necessary small cooking might be accomplished here; dishes and other articles washed, etc.; and it would serve as a general store-room for brooms, pails, and other articles." Although Baron Justus von Liebig (1803–73) boasted that his clinical laboratory performed more than 400 tests per annum, the average mid- to large-sized laboratory nowadays performs several million tests yearly. The term *clinical chemistry* was purportedly coined by Charles Henry Ralfe (1842–96) of London Hospital when he used it as the title of his 1883 treatise. The first laboratory attached to a hospital was established in 1886 in Munich, Germany, by Hugo Wilhelm von Ziemssen. In the United States the first clinical laboratory was The William Pepper Laboratory of Clinical Medicine, established in 1895 at the University of Pennsylvania in Philadelphia.

Molecular diagnostics has more recent origins. "Molecular diagnosis" was first mentioned in 1968 as the title of a *New England Journal of Medicine* editorial, commenting on a new inborn error of metabolism that overproduced oxalic acid, resulting in kidney stones. "Molecular" referred to an enzymatic pathway and the substrates, not nucleic acid variants. Twenty years later, additional articles describing "molecular diagnostics" began to appear. In 1986, molecular diagnostics was defined as, "… the detection and quantification of specific genes by nucleic acid hybridization procedures," exemplified by speciation of plant nematodes. In 1987, molecular diagnostics was used to describe mapping of antigenic substances by affinity chromatography using immobilized antibodies. In 1988 the term was used to describe methods for detecting gene amplification and rearrangement using Southern blotting. With the advent of polymerase chain reaction (PCR), the term "molecular diagnostics" became more common, its use doubling in the medical literature every 6 to 7 years. By 1997, commercial real-time PCR instruments solidified "molecular diagnostics" as a branch of clinical chemistry and laboratory medicine.

BOX 1.1 Uses of Testing in the Clinical Laboratory

- Confirming a clinical suspicion (which could include making a diagnosis)
- Excluding a diagnosis
- Assisting in selection, optimization, and monitoring of treatment
- Providing a prognosis
- Screening for disease in the absence of clinical signs or symptoms
- Establishing and monitoring the severity of a physiological disturbance

EXPANDING BOUNDARIES DEFINED BY TECHNOLOGY

Unlike other specialties in laboratory medicine, clinical chemistry is very much influenced and shaped by technology. No discipline in laboratory medicine uses more technologies than clinical chemistry. Technologies that evolved over time not only changed practice but also remodeled the boundaries of the traditional clinical chemistry laboratory. For example, with the emergence of immunochemical techniques in the 1970s, the US Food and Drug Administration approved many tests for the measurement of proteins, small molecule hormones, and drugs, a development that profoundly changed clinical chemistry and its armamentarium of testing. Integrated automated platforms later enabled the measurement of hormones and therapeutic drugs by immunoassays simultaneously with electrolytes, glucose, and other general chemistry tests, thus subsuming the "endocrine lab" and the "drug lab."

Serologic tests for hepatitis and human immunodeficiency virus (HIV) and tests for autoimmune diseases also moved from their traditional home in microbiology and immunology to chemistry analyzers. Immunoglobulin analysis followed a similar path. The typical clinical chemistry laboratory includes testing for general chemistries, specific proteins and immunoglobulins, therapeutic and abused drugs, blood gases, hormones, biogenic amines, porphyrins, vitamins, and trace elements. Testing for inborn errors of metabolism (such as the measurements of amino acids and organic acids), measurements of coagulation factors, general hematologic testing, and serologic assays can belong either to the clinical chemistry laboratory or to another subspecialty, depending on the institution.

Clinical chemists have embraced technology over the years and used it effectively to derive answers to clinical questions. In modern clinical chemistry laboratories, technologies include spectrophotometry, atomic absorption, flame emission photometry, nephelometry, electrochemical and optical sensor technologies, electrophoresis, and chromatography. The influence of automation, information technology, and miniaturization is evident in current clinical chemistry laboratories. Mass spectrometry, once thought of as a research tool, is playing an ever-growing role in clinical chemistry for the measurement of both small molecules and peptides, and more recently proteins. Point-of-care testing is a disruptive innovation that decentralizes laboratory testing and presents the clinical chemist with many challenges and opportunities.

Molecular diagnostics has forever changed virology and microbiology, introducing faster and more sensitive methods based on nucleic acid amplification rather than microbial replication. Nanotechnology, microfluidics, electrical impedance, reflectance spectroscopy, and time-resolved fluorescence are only a few of the technologies used in point-of-care testing for proteins, drugs, DNA, and analysis of metabolites in small samples of whole blood. Molecular diagnostics in particular impacts diverse specialties, including infectious disease, genetics, and oncology, providing new tools for study at a molecular detail never before considered. In summary, the boundaries of clinical chemistry expand with technology, making the profession vibrant, interesting, and ever evolving.

The scope of the profession is constantly changing for the very same reasons. Scientific and technological developments, medical needs, patient demands, and economic pressures bring various disciplines of medicine closer together, and this integration results in more effective health care. For example, companion diagnostics, which help predict therapeutic responses and individualize patient treatment options, bring together pharmacy and medical laboratories. Point-of-care testing in real time with medical intervention breaks the walls of laboratories to bring the profession closer to clinicians and patients. New disruptive technologies (e.g., "lab on a chip," nanotechnology, home monitoring) as well as movement toward patient empowerment and direct-to-consumer testing bring laboratory testing closer to patients. All of these developments present special challenges to the future generations of laboratory professionals both in terms of how they should be trained and how they will practice.

Technology alone is not the answer to more effective and cost-effective clinical practice. The laboratory data obtained must be meaningful and support clinical management decisions. The generation of more data does not necessarily lead to better patient management and outcomes. In the 1960s and 1970s, with the advent of automated clinical analyzers, laboratories reported chemistry panels of 10 to 20 results. More recently, dense data from expression arrays, genome-wide association studies, epigenomics, and microRNA analyses excel in discovery research, but translation to clinical practice has been slower than anticipated. The promise of greater clinical significance with larger data sets seems intuitive, but history suggests caution. Clinical chemists in this world of "big data" translate high-quality measurement *data* into clinically relevant *information*. This information—when integrated with clinical history and presentation, clinical signs, and an understanding of pathophysiology—becomes *knowledge*. Knowledge, in the context of the experience and judgment of the clinician, is converted to *wisdom* that translates to clinical action for improved patient outcomes.

HOW IS CLINICAL CHEMISTRY PRACTICED?

Although the majority of clinical chemists choose a career in a clinical laboratory environment, many work in the in vitro diagnostics (IVD) and pharmaceutical industries. Clinical chemists, by virtue of their training, are translational researchers who are capable of developing, evaluating, and validating biochemical and genetic assays for clinical use; they develop skills that are essential for new biomarker assays, reagent kits, and companion diagnostics. Clinical chemists also provide interfaces between researchers, clinicians, the clinical laboratory, and the IVD industry to help translate biomarker research into clinically meaningful decisions and actions.

Clinical chemists practicing in the IVD or the pharmaceutical industry may not need to routinely interact with clinicians or interpret laboratory results, but they understand and appreciate the clinical utility and relevance of the assays and companion diagnostics they are developing and thus contribute more effectively to the development of diagnostics that improve health. The daily practice of the profession has changed over time. In the 1960s and 1970s, clinical chemists developed laboratory tests. At present, de novo assay development is still active only in certain areas such as chromatography, mass spectrometry, and molecular diagnostics.

However, as the profession matured and the instrumentation changed from open systems to "black boxes," the traditional analytical focus of the profession has significantly diminished. Clinical chemists are now more active in the preanalytical and postanalytical phases of testing and in establishing processes such as how best to select the right test for the right patient and to communicate test results to clinicians in a medically meaningful way, how to build laboratory processes that reduce error, and how to continuously improve the quality of laboratory practices (Box 1.2).

BOX 1.2 Functions of the Laboratory Professional

- Develop and validate de novo laboratory tests to meet clinical needs.
- Evaluate and characterize the analytical and clinical performance of laboratory tests.
- Present laboratory results to clinicians in an effective manner.
- Provide education and advice on the selection and interpretation of laboratory tests as part of the clinical team.
- Determine the cost effectiveness and intrinsic value of laboratory tests.
- Participate in the development of clinical testing algorithms and clinical practice guidelines.
- Ensure compliance with regulatory requirements.
- Participate in quality assurance and improvement of the laboratory service.
- Teach and train future generations of laboratory specialists.
- Participate in basic or clinical research.

In the current health care environment, there is increasing emphasis on clinical impact and cost effectiveness. Laboratories are expected to demonstrate evidence of improved measurable clinical outcomes and the usefulness and added value of tests to clinical decision making. Proving the fact that laboratory testing contributes to improved patient outcomes is challenging because the relationship between testing and clinical outcomes is mostly indirect. Nevertheless, clinical chemists should move away from being just providers of high-quality data. Transforming laboratory data to information and knowledge requires more skills in information and information management technology, evidence-based medicine, epidemiology, data mining, and translational research. It also requires a shift of thinking from essentialism to consequentialism and from technology-driven to customer-focused and patient-centered laboratory medicine.

To summarize, today's clinical chemists are laboratory professionals who are trained in pathophysiology and technology. The execution of their daily duties, which are more clinically or technology oriented, is influenced by their training (such as MD vs. PhD), interests, institutional needs, and the country where they practice. Clearly the practice of our profession has evolved over the past half a century, and there are even more challenges on the horizon that will expand and change its scope and role and enhance its diversity.

GUIDING PRINCIPLES OF PRACTICING THE PROFESSION

As in other branches of medicine, practitioners in the clinical laboratory are faced with ethical issues, often on a daily basis; examples are listed in Box 1.3.

CONFIDENTIALITY OF GENETIC INFORMATION

Confidentiality of genetic information has been prominent in the news in the first and second decades of this millennium.

BOX 1.3 Ethical Issues in Clinical Chemistry and Molecular Diagnostics

- Confidentiality of genetic information
- Confidentiality of patient medical information
- Allocation of resources
- Codes of conduct
- Publishing issues
- Conflicts of interest

Legislation was considered necessary to prevent denial of health insurance or employment to people found by DNA testing to be at risk of disease. Less appreciated is the fact that the issue of confidentiality of clinical laboratory data predated DNA testing. In fact, many non-DNA tests, old and new, also carry information about risks of illness and death. Clinical laboratory professionals have long been responsible for maintaining the confidentiality of all laboratory results, a situation made even more critical with the advent of increasingly powerful genetic testing.

CONFIDENTIALITY OF PATIENT MEDICAL INFORMATION

New test development requires the use of patient samples and may involve the use of patient medical information. Ethical judgments are required regarding the type of informed consent that is needed from patients for use of their samples and clinical information. Clinical laboratory physicians and scientists often serve on institutional review boards that examine proposed research on human subjects. In these discussions, ethical concepts such as clinical equipoise—which refers to the genuine uncertainty in the expert medical community over whether a particular treatment will be beneficial—and confidentiality are central to decisions.

ALLOCATION OF RESOURCES

Because resources are finite, laboratory professionals must make ethically responsible decisions about allocation of resources. There is often a trade-off between cost and quality. What is best for patients generally? How can the most good be done with the available resources? For laboratorians in business, creative accounting may tarnish the profession if patient care is not kept paramount.

CODES OF CONDUCT

Most professional organizations publish a code of conduct that requires adherence by their members. For example, the American Association for Clinical Chemistry (AACC) has published ethical guidelines that require AACC members to endorse principles of ethical conduct in their professional activities, including (1) selection and performance of clinical procedures, (2) research and development, (3) teaching, (4) management, (5) administration, and (6) other forms of professional service.