PATHOLOGY AND LABORATORY MEDICINE

MOLECULAR DIAGNOSTICS

FOR THE CLINICAL LABORATORIAN

EDITED BY

WILLIAM B. COLEMAN
GREGORY J. TSONGALIS

分子诊断学临床实验指南

世界图书出版公司

HUMANA PRESS

Molecular Diagnostics

For the Clinical Laboratorian

世界图书出版公司

西安 北京 广州 上海

(陕)新登字 014 号

陕版出图字　著作权合同登记　25-1999-018 号

Molecular Diagnostics
—For the Clinical Laboratorian
分子诊断学—临床实验指南

by William B. Coleman et al.
任卫军　重印责任编辑
世界图书出版西安公司　重印发行
(西安市 南大街 17 号　邮编 710001)
西安七二二六印刷厂印刷
37 × 1092 毫米　开本 1/16　印张 25
1999 年 6 月第 1 次重印
ISBN 7 - 5062 - 2239 - 6/R·377
定价:150.00 元

999 Riverview Drive, Suite 208
Totowa, New Jersey 07512

For additional copies, pricing for bulk purchases, and/or information about other Humana titles, contact Humana at the above address or at any of the following numbers: Tel.: 201-256-1699; Fax: 201-256-8341; E-mail: humana@mindspring.com

This publication is printed on acid-free paper.
ANSI Z39.48-1984 (American Standards Institute) Permanence of Paper for Printed Library Materials.

Cover illustration: From Fig. 6 in Chapter 2 "An Overview of Nucleic Acid Chemistry, Structure, and Function: *The Foundations of Molecular Biology,*" by William B. Coleman.

Cover design by Patricia F. Cleary.

Printed in the United States of America. 10 9 8 7 6 5 4 3 2 1

Library of Congress Cataloging-in-Publication Data

Molecular diagnostics : for the clinical laboratorian / edited by
William B. Colaman and Gregory J. Tsongalis.
p. cm.
Includes index.
ISBN 0-89603-373-2 (alk. paper)
1. Molecular diagnosis, 2. Pathology, Molecular. I. Coleman.
William B. II. Tsongalis, Gregory J.
[DNLM: 1. Technology, Medical. 2. Genetic Techniques.
3. Diagnosis, Laboratory—methods. 4. Molecular Biology—methods.
5. Chemistry, Clinical—methods. WB 365 M718 1997]
RB43/7.M65 1997
616.07'56—dc21
DNLM/DLC
for Library of Congress 96-37505
CIP

Molecular Diagnostics

For the Clinical Laboratorian

Edited by

William B. Coleman

University of North Carolina, Chapel Hill, NC

and

Gregory J. Tsongalis

Hartford Hospital, Hartford, CT

Humana Press  **Totowa, New Jersey**

Preface

The clinical laboratory has long functioned as a dynamic environment in which new technologies constantly challenge us by their implementation in new diagnostic tests. With the goal of providing the most sensitive and specific tests for monitoring disease, the clinical laboratory is now challenged by yet another technological advancement, that occasioned by the development and applications of molecular biology to the analysis of nucleic acids. It is already clear that this will have a most significant impact on the clinical laboratory with respect to the sensitivity/specificity of tests as well as their broad application to all areas of laboratory medicine. The information provided by molecular diagnostic testing will change the way laboratory medicine is practiced in an unprecedented manner.

The success of this molecular diagnostic approach in a clinical setting is highly dependent on the training of well-qualified technologists, residents, and clinicians alike, who will not only have to perform these tests and interpret their results, but also understand the limitations of both the technology and the results. We address these issues in chapters specifically dealing with each technique, and in subsequent chapters include applications to the diagnosis of various forms of disease. This book is intended for use by medical technologists, residents, fellows, and clinicians of all medical disciplines who will be affected by one or more applications of this technology to any given subspecialty.

It has been a mere ten years since this technology shifted from the research arena to the clinical arena. In part because of the success of the Human Genome Project, both the technology and its applications are being introduced to the medical community at record-setting speeds. Our goal is to present this revolutionary technology to these trainees in a concise yet understandable fashion and include examples of its applications to the various divisions of laboratory medicine as an indicator of what the future of diagnostic medicine will be.

William B. Coleman
Gregory J. Tsongalis

Dedications

Over the years, many individuals have played crucial roles in our successes, but none greater than those members of our families who gave continuous support in an unselfish manner.

This book is dedicated to our parents, Alice and Byrns Coleman and Mary and Demetrios Tsongalis for believing in higher education and helping make dreams come true.

To our wives, Monty and Nancy, for their unprecedented support, understanding, and appreciation for what we do.

To our children, brothers, sisters, and extended families for the many years of love, friendship, and tolerance.

W. B. C.

G. J. T.

Contributors

EVELYN C. ABERNATHY • *Department of Pathology and Laboratory Medicine, Hartford Hospital, Hartford, CT*

JABER ASLANZADEH • *Department of Laboratory Medicine, University of Connecticut School of Medicine, Farmington, CT*

JEFFREY S. BARTLETT • *Gene Therapy Center, University of North Carolina School of Medicine, Chapel Hill, NC*

BRUCE F. BOWER • *Department of Clinical Endocrinology, Hartford Hospital, Hartford, CT; University of Connecticut School of Medicine, Farmington, CT*

WILLIAM B. COLEMAN • *Department of Pathology and Laboratory Medicine, University of North Carolina School of Medicine, Chapel Hill, NC*

JOHN H. CONTOIS • *Department of Pathology and Laboratory Medicine, Hartford Hospital, Hartford, CT*

MATTHEW S. COWLEN • *Department of Pathology and Laboratory Medicine, McLendon Clinical Laboratories, University of North Carolina Hospitals, Chapel Hill, NC; Present Address: Pathology Associates International, Frederick, MD*

PAMELA A. CRIFASI • *Department of Laboratory Medicine, Molecular Genetics Laboratory, Mayo Clinic, Rochester, MN*

R. KEITH ESCH • *Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC. Present Address: Department of Biology, Ursinus College, Collegeville, PA*

DANIEL H. FARKAS • *Department of Clinical Pathology, William Beaumont Hospital, Royal Oak, MI*

W. EDWARD HIGHSMITH, JR. • *Department of Pathology, University of Maryland, Baltimore, MD*

JUCH CHIN HUANG • *Department of Pathology and Laboratory Medicine, Hartford Hospital, Hartford, CT*

CARL D. MALCHOFF • *Department of Medicine and Surgery, Surgical Research Center, University of Connecticut Health Center, Farmington, CT*

ROBERT E. MOORE • *Department of Pathology and Laboratory Medicine, Hartford Hospital, Hartford, CT*

SHARON COLLINS PRESNELL • *Department of Pathology and Laboratory Medicine, University of North Carolina School of Medicine, Chapel Hill, NC*

WILLIAM N. REZUKE • *Department of Pathology and Laboratory Medicine, Hartford Hospital, Hartford, CT*
ELIZABETH M. ROHLFS • *Department of Pathology and Laboratory Medicine, McLendon Clinical Laboratories, University of North Carolina Hospitals, Chapel Hill, NC*
LAWRENCE M. SILVERMAN • *Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC*
ROBERT B. SISK • *Department of Marine Science, University of North Carolina School of Medicine, Chapel Hill, NC*
DAVID H. SORSCHER • *Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC*
STEPHEN N. THIBODEAU • *Department of Laboratory Medicine, Mayo Clinic, Rochester, MN*
GREGORY J. TSONGALIS • *Department of Pathology and Laboratory Medicine, Hartford Hospital, Hartford, CT*
ZHENYUAN WANG • *Department of Laboratory Medicine, Molecular Genetics Laboratory, Mayo Clinic, Rochester, MN*
MYRA J. WICK • *Department of Laboratory Medicine, Molecular Genetics Laboratory, Mayo Clinic, Rochester, MN*

Contents

Part I

Basic Molecular Biology

1

A Historical Perspective on the Clinical Diagnostic Laboratory

Robert E. Moore

1. INTRODUCTION

As the clinical diagnostic laboratory prepares to enter the 21st century, it is interesting to reflect on past scientific and social events that have influenced the current status of the laboratory, and anticipate future problems and opportunities. It would be a mistake to suggest that all the significant medical and social events that had an impact on the present laboratory function can be discussed or evaluated in a short introductory chapter. It is also possible to mistakenly attribute more influence to some events than they deserve simply because they are being interpreted with a 20th century bias. However, some threads of commonality have influenced the evolution of diagnostic laboratories from earliest times. It is the objective of this chapter to highlight events that, in my opinion, have shaped how and/or why clinical laboratories have arrived at their present position in the practice of medicine.

A history of diagnostic testing can be started by reviewing the evolution of diagnostic tests from isolated procedures to organized diagnostic laboratory testing. Originally, laboratory tests were performed at the side of the patient with small, simple equipment, rapid evaluation of the result, and a diagnostic opinion rendered. Test choice, performance, and interpretation were all left to the individual practitioner. There was no professional support staff to assist at any point in the process. Little exchange took place between practitioners, because the individual's success was directly related to which procedures were done and the manner in which they were done. A premium was paid for showmanship as well as successful treatments.

The modern laboratory is a physical place, either standing alone or as a component of a health care institution, with numerous pieces of complicated capital equipment, where hundreds and sometimes thousands of specimens per day are processed for dozens of tests. Usually the laboratory accepts a teaching and a research responsibility to accompany this patient service obligation. Today's laboratories are staffed by professionals trained in several subspecialities, who are available for consultation in all aspects of diagnostic testing. This structure underlines the current complexity and sophistication of the modern laboratory operation. There are a myriad of professional organizations, meetings, and publications that have developed to make information

From: Molecular Diagnostics: For the Clinical Laboratorian
Edited by: W. B. Coleman and G. J. Tsongalis Humana Press Inc., Totowa, NJ

exchange convenient and efficient. This exchange is also necessary to ensure a single level of care. The system that supports showmanship and theatrics has been replaced by one that only tolerates accepted medical practice.

Today's laboratory is in a state of flux, faced with issues of near-patient testing, outreach programs, utilization, extensive regulation, and stringent fiscal controls. The first three of these issues are reminiscent of the early beginnings of diagnostic testing. The laboratory organization has replaced the individual practitioner, but testing is moving back toward the patient in the form of near patient testing or outreach programs. In the past, a few individuals spoke about appropriate use of diagnostic testing, whereas today committees and organizations are dedicated to the control of laboratory utilization—an ontogeny recapitulating phylogeny phenomenon. A review of history gives some insight into relationships and similarities of past activity with current practice. What are perceived as new problems and opportunities can be traced to antiquity.

The development of the modern laboratory required several conditions to be met at appropriate times in history. Obviously, technology was and is the primary force behind advances in medicine. This has been both a blessing and a curse. It is a blessing because the understanding and treatment of disease require sophisticated tools that only technological advances can produce. It can be a curse because there is a direct relationship between sophistication and health care cost. Other social issues dealing with availability and ethics assume a much greater importance than in the past. Angiography, CAT scans, organ transplantation, and DNA analysis are just a few of the expensive but valuable technologies available to the modern physician. Such questions as when are they used, to whom are they available, and what is done with the result, are dilemmas of the 20th-century laboratory.

Another result of this technology is that it removes a large number of tests and procedures from the primary physician. The expertise required to perform these and other procedures combined with the significant cost of the equipment precludes the primary physician from being the laboratorian.

A second condition that had to be met was logistics. Laboratory testing had to be convenient. All the concerns of collection, preservation, testing, and reporting had to be easy and fast for both patient and physician. A practical consequence of this was the proliferation of laboratories with increased resources to deliver the service. In the beginning, these resources were human and consisted of having specimens transported to the primary laboratory site. This later became multiple collection sites, courier services, mechanical processing, and electronic reporting.

These logistical issues lead directly to a third condition for laboratory development: economics. There are two components to economic considerations. First, the laboratory service has to be an economically viable option for the ordering physician. The service must be delivered in such a way that the physician does not experience any cost or significant loss of income from referring tests to a diagnostic laboratory. Second, there must be a mechanism to support the cost of laboratory testing. This latter problem was solved, for a short time, by the third-party reimbursement system. As one of the social reform programs of the 20th century, the widespread availability of insurance made the cost of health services invisible to the patient. Without proper controls, the effect of this reimbursement system was to encourage the proliferation of technology and make service accessible to large

segments of the population. The incentive was for every testing center to have all the best technology and make it available to everyone.

This chapter will highlight some historical events and practices that demonstrate how this evolution took place. The events outlined here are not absolute in defining the practice of laboratory medicine, but suggest how concepts could evolve and develop into the practice of diagnostic laboratory testing as it exists today.

2. EARLIEST MEDICINE

Long before there were laboratories there were accepted practices for patient evaluation. Early health care providers (not all were physicians) attempted to determine the health status of the person under evaluation by any means possible. The diagnosis and the prescribed treatment were not always an accurate or scientifically based pronouncement. It was a process that was motivated by a combination of altruism, vanity, greed, scientific thought, and philosophical and religious edict. This is not meant to imply that all was quackery and incantation. The procedures that had medical value are the ones that laid the foundation for legitimizing diagnostic medicine along with its subsequent support functions, one of which is the clinical diagnostic laboratory.

One major obstacle that physicians faced in ancient times was that it was illegal to practice invasive procedures. The patient could be observed and touched, but the only specimens that could be taken were those that naturally passed from the body. As a result of these limitations, urine has been the sample with the longest history of evaluation. There is some evidence that the Sumerians and Babylonians used urine for diagnosis as early as 4000 BCE *(1)*. The diagnosis of pregnancy was probably made by ancient Egyptians using the urine of the woman to germinate seeds *(1)*. Hindu medicine describes the sweet taste of urine and that black ants are attracted to this urine if it is poured on the ground *(1)*. Hippocrates (460–355 BCE) described the characteristics and colors of urine from his patients, and mentioned bubbles being present in urine from patients with long-standing kidney disease *(1–3)*. Over the next 600 years, the study of urine was advanced very little. Galen (129–200 AD) wrote and taught that urine was a filtrate of blood, and as such, could indicate the type and location of illnesses *(1)*. The teachings of these two men were the information base for urinalysis, or uroscopy, as it was called, for the next 9–10 centuries.

During this time, approx 800 AD, the first treatise on urine was written by Theophilus Protospatharius; in it is mentioned the first chemical test done on urine. The urine from patients with kidney disease was heated over a candle flame and became cloudy *(1)*. Other physicians repeated the process, some substituting acid for heat, and although it took centuries before the precipitating substance was identified as protein, the association with disease had been recognized *(3)*.

Other observations were made concerning the quality of the urine sample. The differences between morning and afternoon urine samples and factors like age, food, and medicaments exerted effects on the composition of urine. These were noted as early as the 10th century by Avacinna *(1)*. It was realized as early as the 11th century that the first voided specimen in the morning was the best urine specimen for analysis, and that when 24-h urine collections were required, they should be protected from light and heat *(1)*.

Urinalysis continued to be such a focus of study that Gilles de Corbeil developed a glass vessel (called a matula), shaped like a urinary bladder, specifically for the exami-

nation of urine. The concept was that the urine sediments and discoloration would occur in the vessel at a place that corresponded to the site of pathology in the body. These early urine vessels were among the first pieces of laboratory equipment and were so common and identifiable that they were one of the predecessors to the caduceus as the symbol of medicine.

Other tests and procedures were added to urinalysis and various aides were developed to make the process easier. One device of this type was the urine wheel. This wheel was the original color chart similar in purpose to those that accompany most modern dipstick packages. Along with matching the urine color on the wheel, there was an interpretive text included to assist in making a diagnosis *(3)*.

Uroscopy soon took on a life of its own. Samples were sent to physicians without any explanation of the patient's complaint, and it was expected the physician would return a diagnosis and therapy *(3)*. Expectedly, uroscopy was ripe for abuse, and assuredly this happened. The prominence of the practitioner was enhanced if he was perceived as being able to do more than other uroscopists. Consequently, claims were made concerning the interpretive powers of the analyst that far exceeded the limits of observation. It was during this period, when there was an opportunity to make a handsome income from urine analysis, that Joannes Actuarius began to write about the limitations of urine examination. He was one of the first to caution that urine examination, independent of how well it was done, could not be used to the exclusion of all other clinical findings *(3)*.

It should not be inferred that uroscopy was always a questionable effort. Proteinuria, although protein as such had not been identified, nephritis, type I diabetes, hematuria, infection, concentration, and limited assessment of liver disease were all recognized through urinalysis. In most cases it took centuries to identify the specific component in urine and its association with a disease. Nevertheless, urine testing was a valuable diagnostic tool *(3)*.

These early attempts to diagnose disease through the study of the only practical body fluid available gives some insight into the development of laboratory medicine. First, the progression from pure observation to the use of some elementary aids and procedures suggests that the seed of intellectual curiosity was germinating. If simple observation was useful, then employing procedures to define components was better. Second, equipment was being generated for a specific medical application. The equipment was primarily small and portable, but with such procedures as distillation, precipitation, and evaporation, the need for a place to do this work was becoming an issue. A permanent site or address for the practitioner to do laboratory work enabled patients to "send" their urine for analysis (an early precursor to the outpatient laboratory). Third, since uroscopy was becoming a routine practice, some members of the legitimate medical community were discussing the appropriate use of these procedures. This may be the first suggestion of test utilization in history.

3. THE TRANSITION

Progress requires an advance in technology or the appearance of a gifted individual. Science and technology had advanced to a point where a breakthrough on some other front was required. Two historic events that meet this requirement are the invention of movable type by Johann Gutenberg in the 15th century and the Reformation. Moveable