

Molecular Diagnostics

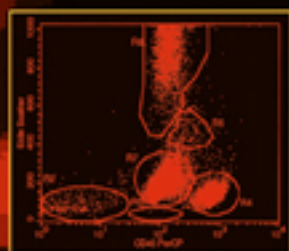
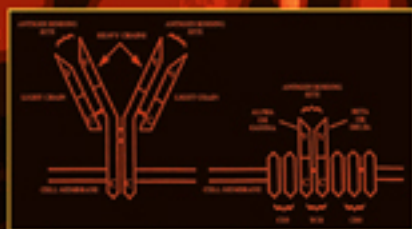
For the Clinical Laboratorian

Edited by

William B. Coleman

Gregory J. Tsongalis

SECOND EDITION



HUMANA PRESS

MOLECULAR DIAGNOSTICS

MOLECULAR DIAGNOSTICS

For the Clinical Laboratorian

Second Edition

Edited by

WILLIAM B. COLEMAN

*Department of Pathology and Laboratory Medicine
University of North Carolina School of Medicine
Chapel Hill, NC*

and

GREGORY J. TSONGALIS

*Department of Pathology
Dartmouth Medical School
Dartmouth-Hitchcock Medical Center
Lebanon, NH*

Foreword by

LAWRENCE M. SILVERMAN

*Department of Pathology and Laboratory Medicine
University of Virginia
Charlottesville, VA*



HUMANA PRESS

Editors

William B. Coleman
Department of Pathology and
Laboratory Medicine
University of North Carolina
School of Medicine
Chapel Hill, NC

Gregory J. Tsongalis
Department of Pathology
Dartmouth Medical School
Dartmouth-Hitchcock Medical Center
Lebanon, NH

ISBN 978-1-58829-356-5 e-ISBN 978-1-59259-928-8
DOI 10.1007/978-1-59259-928-8
Springer New York Dordrecht Heidelberg London

Library of Congress Cataloging-in-Publication Data

© Humana Press, a part of Springer Science+Business Media, LLC 2006

All rights reserved. This work may not be translated or copied in whole or in part without the written permission of the publisher (Humana Press, c/o Springer Science+Business Media, LLC, 233 Spring Street, New York, NY 10013, USA), except for brief excerpts in connection with reviews or scholarly analysis. Use in connection with any form of information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed is forbidden.

The use in this publication of trade names, trademarks, service marks, and similar terms, even if they are not identified as such, is not to be taken as an expression of opinion as to whether or not they are subject to proprietary rights.

While the advice and information in this book are believed to be true and accurate at the date of going to press, neither the authors nor the editors nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Printed on acid-free paper

(Corrected at 4th printing 2010)

Springer is part of Springer Science+Business Media (www.springer.com)

DEDICATION

First and foremost, we would like to dedicate this book to our wives, Monty and Nancy, for their unprecedented support of our careers and families.

To all of our colleagues, students, trainees, and mentors over the many years, we thank you for your continued support.

FOREWORD

In 1994 I coedited a book, *Molecular Pathology: Approaches to Diagnosing Human Disease in the Clinical Laboratory*, that coined the phrase “molecular pathology” to refer to applications of molecular biology in the traditional areas of laboratory medicine. That compilation of clinical molecular techniques included 11 chapters and an epilogue on “New Directions for the Clinical Laboratory.” Chapter headings included: automation, neoplasia, heritable diseases, and infectious diseases.

Three years later, in 1997, Bill Coleman and Greg Tsongalis edited *Molecular Diagnostics: For the Clinical Laboratorian*, which contained 18 chapters and introduced the new areas of RT-PCR and *In Situ* PCR, and included a section on gene therapy. Now, ten years after the introduction of molecular pathology, we are looking at the second edition of Bill Coleman and Greg Tsongalis’ text.

This second edition now comprises 44 chapters, and contains new chapters on bioinformatics, microarrays, methylation assays, FISH, laser capture microdissection, quality assurance, chimerism studies for bone marrow transplantation, and separate chapters on genetic counseling and ELSI (ethical, legal, and social issues). These new chapters represent developments in the past decade that have fundamentally changed the scope from molecular pathology to molecular diagnostics, highlighting the changing role of the clinical laboratorians who direct these

efforts. It should be evident that molecular pathology and diagnostics impact almost every conceivable subspecialty in laboratory medicine and, particularly in the case of pharmacogenomics, have led to the development of new areas of investigation.

What is less evident is the burden placed upon the laboratorians directing these efforts. Issues regarding training, certification, continuing education, and reimbursement (just to name a few) have demanded enormous time and effort from professional organizations and governmental agencies. In addition, because conventional approaches cannot always be applied to molecular diagnostics, unique solutions for quality assurance and quality control must be developed. These challenges have engendered committees, subcommittees, taskforces, and workgroups from regulatory agencies and professional organizations, representing worldwide constituencies. As in any situation involving so many players, consensus-building and communication are mandatory. To these ends, professional journals and textbooks are our best hope for remaining current with this rapidly changing field. One needs only to compare the wealth of knowledge in this current edition with our efforts of only ten years ago to appreciate the magnitude of this challenge.

Lawrence M. Silverman, PhD

PREFACE

It has been almost ten years since the concept for producing the first edition of *Molecular Diagnostics: For the Clinical Laboratorian* was conceived. In those ten years the field of molecular pathology and diagnostics has exploded as many predicted. The clinical diagnostic laboratory continues to function as the playing field for this expansion that includes vast and dynamic changes in test menus, instrumentation, and clinical applications. The impact of this field on the routine practice of clinical medicine and management of patients continues to be felt as new developments that span all areas of laboratory medicine exceed our expectations.

The success of this technology in a clinical setting is highly dependent upon the training of well-qualified technologists, residents, and clinicians alike, who will not only have to perform and interpret results of these tests but also understand the limitations of the technology and resulting clinical implications. The production of this second edition is a testament to our passion and commitment for the teaching and training of qualified individuals who wish to embark on this journey. The numerous training programs, educational venues, and board certification examinations that have evolved over the past ten years also sends a strong vow of commitment by others in the field to ensure the successful use of these new tools in supporting the best possible patient care that is available.

The second edition of *Molecular Diagnostics: For the Clinical Laboratorian* begins with a historical perspective of laboratory medicine followed by an overview of basic molecular biology techniques and concepts. Part III provides a more in depth examination of some advanced molecular technologies and their potential uses. Part IV describes other technologies found in the clinical laboratory that can complement or be complemented by molecular diagnostic technologies. The increasing need for awareness and practice of quality assurance in this field led us to include a complete section (Part V) that examines some of these issues. Although the first edition included clinical applications all in one section, the increased number of applications led us to develop separate sections for genetic disease, human cancers, infectious diseases, and identity testing (Parts VI–IX). Finally, the book concludes with a section on genetic counseling and ethical/social issues involved with nucleic acid testing.

Although no such book could possibly be all encompassing in such a rapidly developing field, we feel that the material covered herein will provide the reader with an excellent overview.

William B. Coleman
Gregory J. Tsongalis

CONTENTS

Dedication	v
Foreword	vii
Preface	ix
Contributors	xv
Part I. Introduction	
1 An Historical Perspective on the Clinical Diagnostic Laboratory	3
<i>Robert E. Moore</i>	
Part II. Basic Molecular Biology	
2 An Overview of Nucleic Acid Chemistry, Structure, and Function: <i>The Foundations of Molecular Biology</i>	13
<i>William B. Coleman</i>	
3 Extraction of Nucleic Acids	25
<i>Paul N. Bogner and Anthony A. Killeen</i>	
4 Nucleic Acid Blotting Techniques: <i>Theory and Practice</i>	31
<i>Terry Amiss and Sharon Collins Presnell</i>	
5 The Polymerase Chain Reaction	47
<i>William B. Coleman and Gregory J. Tsongalis</i>	
6 Bioinformatics: <i>Computer-Based Approaches to Genetic Analysis</i>	57
<i>Sharon L. Ricketts</i>	
Part III. Molecular Diagnostic Technologies	
7 PCR-Based Methods for Mutation Detection	65
<i>Ian M. Frayling, Emma Monk, and Rachel Butler</i>	
8 Alternative Methods for Amplified Nucleic Acid Testing	75
<i>Deborah A. Payne and Laurie E. Sower</i>	
9 Electrophoretic Methods for Mutation Detection and DNA Sequencing	85
<i>W. Edward Highsmith, Jr.</i>	

10	Single-Nucleotide Polymorphisms: <i>Testing DNA Variation for Disease Association</i>	111
	<i>Ulrich Broeckel and Martin J. Hessner</i>	
11	Microarray Approaches to Gene Expression Analysis	121
	<i>David Neil Hayes and Matthew Meyerson</i>	
12	Methods for Analysis of DNA Methylation.....	149
	<i>Alexander Dobrovic</i>	
Part IV. Other Clinical Diagnostic Technologies		
13	Flow Cytometry	163
	<i>Joseph A. DiGiuseppe</i>	
14	Medical Cytogenetics	173
	<i>Martha B. Keagle</i>	
15	Fluorescence <i>In Situ</i> Hybridization: <i>A Major Milestone in Luminous Cytogenetics</i>	189
	<i>Suneel D. Mundle and Robert J. Koska</i>	
16	Immunohistochemistry	203
	<i>John Hunt, Larissa Davydova, Richard W. Cartun, and Maria Baiulescu</i>	
17	Laser Capture Microdissection	219
	<i>C. Robert Bagnell, Jr.</i>	
Part V. Quality Assurance in the Molecular Diagnostics Laboratory		
18	Framework for Quality Assurance in Molecular Diagnostics	227
	<i>Marlene Sabbath-Solitare, Selwyn J. Baptist, and Teresita Cuyegkeng Redondo</i>	
19	Verification of Molecular Assays.....	237
	<i>Brent L. Seaton</i>	
20	Standards and Standardization of Molecular Diagnostics	243
	<i>John P. Jakupciak and Catherine D. O'Connell</i>	
21	Laboratory-Developed Tests in Molecular Diagnostics	247
	<i>Andrea Ferreira-Gonzalez and Carleton T. Garrett</i>	
Part VI. Applications of Molecular Diagnostics for Genetic Diseases		
22	An Overview of Molecular Genetics.....	259
	<i>Elaine Weidenhammer and Gregory J. Tsongalis</i>	
23	Genetic Basis of Neurologic and Neuromuscular Diseases	267
	<i>Narasimhan Nagan, Christopher J. Klein, D. Brian Dawson, Myra J. Wick, and Stephen N. Thibodeau</i>	

24	Molecular Mechanisms of Endocrine Disorders	281
	<i>Amy Potter and John A. Phillips III</i>	
25	Molecular Pathogenesis of Cardiovascular Disease	295
	<i>Alan H. B. Wu</i>	
26	Molecular Diagnostics in Coagulation	311
	<i>Enrique Ballesteros</i>	
27	Cystic Fibrosis	321
	<i>Eugene H. Lewis III, Myra J. Lewis, Jean A. Amos, and Gregory J. Tsongalis</i>	
28	Prenatal Genotyping for Identification of Fetuses at Risk for Immune Cytopenic Disorders	329
	<i>Martin J. Hessner and Brian R. Curtis</i>	
29	Personalized Medicine	341
	<i>Karen McCullough</i>	

Part VII. Applications of Molecular Diagnostics for Human Cancers

30	Molecular Pathogenesis of Human Cancer	349
	<i>William B. Coleman and Gregory J. Tsongalis</i>	
31	Application of Molecular Diagnostics to Hereditary Nonpolyposis Colorectal Cancer	375
	<i>Ian M. Frayling, Lisa Happerfield, Christopher Mattocks, Kim Oakhill, and Mark J. Arends</i>	
32	Molecular Genetic Applications to the Diagnosis of Lymphoma	393
	<i>William N. Rezukey, Jr. and Gregory J. Tsongalis</i>	
33	Molecular Genetic Abnormalities in Acute and Chronic Leukemias	415
	<i>Pei Lin and L. Jeffrey Medeiros</i>	

Part VIII. Applications of Molecular Diagnostics for Infectious Diseases

34	Molecular Testing for <i>Chlamydia trachomatis</i> and <i>Neisseria gonorrhoeae</i>	437
	<i>Alexandra Valsamakis</i>	
35	Human Papillomavirus	447
	<i>Theresa M. Voytek and Gregory J. Tsongalis</i>	
36	Molecular Diagnosis for HIV-1	453
	<i>Angela M. Caliendo and Charles E. Hill</i>	
37	Hepatitis C	461
	<i>D. Robert Dufour</i>	

38 Cytomegalovirus	473
<i>Angela M. Caliendo and Nell S. Lurain</i>	
Part IX. Applications of Molecular Diagnostics for Identity-Based Testing	
39 HLA Typing Using Molecular Methods	485
<i>John L. Schmitz</i>	
40 Molecular Analysis for Forensic Casework and Parentage Testing	495
<i>Tracey Dawson Cruz</i>	
41 Molecular Assessment of Bone Marrow Transplant Engraftment	511
<i>Jessica K. Booker</i>	
42 Use of DNA-Based Identity Testing for Specimen Identification	517
<i>Andrew Ricci Jr. and Gregory J. Tsongalis</i>	
Part X. Issues for the Clinical Molecular Pathology Laboratory	
43 Genetic Counseling Considerations in Molecular Diagnosis	525
<i>Myra I. Roche</i>	
44 Ethical, Social, and Legal Issues Related to Molecular Genetic Testing	545
<i>Jennifer L. Herbst and Jon F. Merz</i>	
Index	555