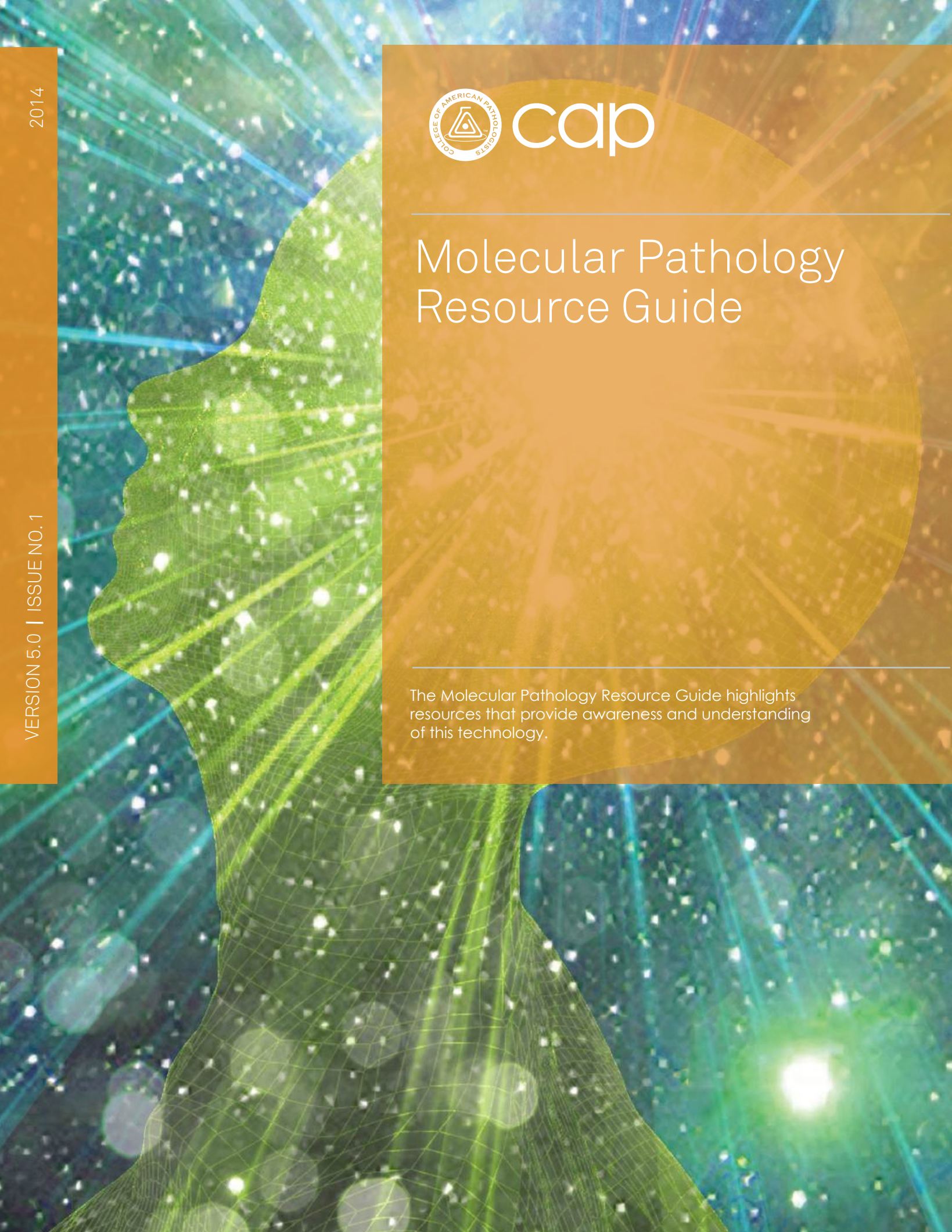

2014

VERSION 5.0 | ISSUE NO. 1

Molecular Pathology Resource Guide

The Molecular Pathology Resource Guide highlights resources that provide awareness and understanding of this technology.

Goal of this Resource Guide and How to Use It

What is the Resource Guide?

The Molecular Pathology Resource Guide is one of four CAP Resource Guides that brings a collected set of resources together in one place that are focused on a specific hot-topic technology important to pathologists. Each comprehensive guide highlights current resources such as a curated set of journal articles, and a collected set of CAP resources that includes learning opportunities, proficiency testing, and accreditation related to this technology. Also, each Resource Guide includes an “Insights From Adopters” section to gain perspective from pathology leaders in the field. In sum, each Resource Guide provides a one-stop resource that will assist busy pathologists to find valuable information about a dynamic and important emerging technology.

How to Use This Molecular Pathology Resource Guide

This Resource Guide is designed in a modular manner to facilitate its use in several different ways. For example, the guide may be used in its entirety as a comprehensive guide to the rapidly evolving field of molecular pathology. Conversely, it may be used by a pathologist to focus on and gain a current understanding of the application of molecular pathology to a very specific organ system or disease process. The tables are designed not only, to organize and summarize the contents of a section, but also to serve as stand-alone, quick reference guides to a topic. To some, these tables may hold the greatest value and become a frequently used reference. The Adopters sections will undoubtedly prove to be of great value to those contemplating taking or actually taking the plunge into the enhanced application of molecular pathology approaches to their practice.

Goal of Guide

Special Features of the Molecular Pathology Resource Guide

Be sure and see the Quick Reference Table: Genes by Tumor Type in Section 1.2. Note that there are other valuable tables such as Target Genes (Section 3.2.2), Genes of Prognostic and Diagnostic Significant (Section 3.8), and Commonly Tested Genes for Hereditary Disease (Section 4.2.1) that you will find of value. Be sure and see Section 8.1 on the SPECs- Short Presentations in Emerging Concepts which provides a valuable tool for tumor boards or in discussion with local clinicians about emerging molecular tests that are actionable for patient care today. Also, note, the CAP webinars series on genomic and molecular topics- listed in Section 8.2.1 and Section 8.2.2.

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The Molecular Pathology Resource Guide is a product of the CAP CSA's Personalized Health Care Committee.

The CAP has four Pathology Resource Guides:
Pathology Resource Guide: Genomics
Pathology Resource Guide: Molecular Pathology

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Goal of Guide

Pathology Resource Guide: Digital Pathology
Pathology Resource Guide: In Vivo Microscopy

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Section 8 CAP Molecular Diagnostics Education Resources

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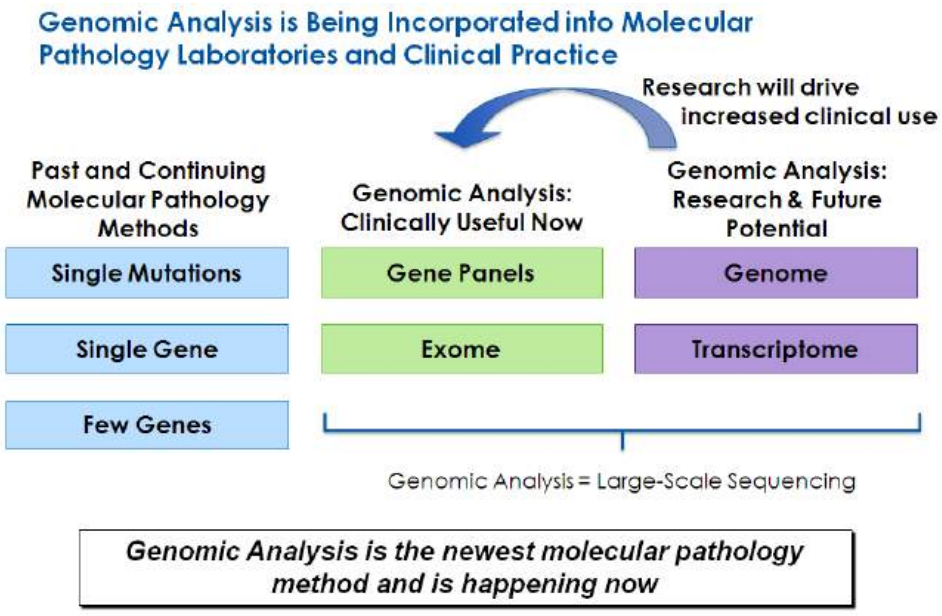
Section 1 Molecular Diagnostics: The Basics

1.1 Background

Molecular testing is a well established yet rapidly developing field of pathology. Pertaining to the diagnostics of DNA, RNA and protein, it assists clinicians and pathologists alike in the diagnosis, prognosis and theranosis of human disease states. This resource guide is designed to discuss the currently established role of molecular testing in medicine, namely the testing of single mutations, single genes or small panels of genes. More extensive analysis of the genome, aka genomic analysis, is a developing field of pathology and is discussed in the Genomic Analysis Resource Guide, available at www.cap.org.



*The diagram above depicts the broad spectrum of molecular/genomic analysis. The focus of this resource guide is molecular testing, circled in red

Current molecular testing covers four major arenas: infectious disease (i.e. respiratory virus detection), hereditary disease (i.e. cystic fibrosis carrier screening), oncology (i.e. EGFR mutation analysis in lung

adenocarcinoma) and pharmacogenomics. With regards to relative volumes of these types of testing, infectious disease molecular diagnostics is more frequently requested than hereditary disease testing which is more frequent than oncologic testing. There are many laboratory operation similarities between all three arenas, such as contamination prevention, lab space requirements, and methods; however each arena has operational differences as well. For example, labs performing infectious disease testing will likely have larger volumes and opportunities for automation should be considered. A lab performing hereditary disease testing must be concerned with proper informed consent, consistent with state and federal regulations and standards. And finally, labs performing oncologic molecular testing must establish proper techniques for FFPE slide selection, documentation and/or micro dissection.

Financial considerations must also be thoroughly evaluated when considering molecular testing. Decisions to offer a test “in-house” instead of sending it to a reference lab , determining a tests value to the clinical scenario, and the utilization of laboratory developed tests (LDT’s) vs. FDA approved *in vitro* diagnostics (IVD’s) are just an example of questions to be answered by a pathologist considering molecular testing.

This resource guide is designed to be a succinct source of information regarding molecular testing and to provide references to assist a pathologist exploring molecular diagnostics.

1.2 Selecting Which Molecular Test-Where to Start – Quick Reference Table: Genes by Tumor Type

A) Clinical Requests for Molecular Tests: The 3-Step Evidence Check

Carter AB. Clinical Requests for Molecular Tests: The 3-Step Evidence Check. *Archives of Pathology & Laboratory Medicine*: December 2012, Vol. 136, No. 12, pp. 1585-1592. http://dx.doi.org/10.5858/arpa.2011-0691-SA

Summary: Laboratory tests performed by molecular methods are increasing in volume and complexity at an unprecedented rate. Molecular tests have a broad set of applications, and most recently have been advocated as the mechanism by which providers can

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further tailor treatments to the individual patient. As the momentum behind molecular testing continues to increase, pathology practices may find themselves unprepared for the new wave of molecular medicine. This special article has been developed in an effort to provide pathologists who have limited molecular training with a simple and quick algorithm for determining whether a requested molecular test is appropriate for a patient. Additional recommendations for a more intensive and proactive review and management of molecular requests also are included. The principles discussed can easily be applied to requests for any test, including those not using molecular methods, which would be sent to an outside reference laboratory. This special article was developed from a Webinar for the College of American Pathologists targeting education for pathologists about the transformation of pathology practice in the new molecular and digital age.

Free full free text from the CAP's *Archives*
PMID: 22480222
NOTE: Also cited in Section 2.1

B) Personalized Medicine: Framing the Issues for Pathology
Leonard D. PHC Webinar. Personalized medicine: framing the issues for pathology. [Webinar]. April 23, 2010. https://www1.gotomeeting.com/register/636002000. Accessed December 8, 2011.

Summary: Talk of Personalized Medicine is everywhere: in the newspapers, in the scientific and medical literature, on the internet. There are companies offering "recreational genomics" testing directly to consumers. But Personalized Healthcare (PHC) is not discussed very much in your doctor's office or in our Pathology Laboratories. Are the trends in genomics a threat or an opportunity for pathologists? Why should pathologists care about the discussions of Personalized Medicine? Can Pathologists hope this will all blow over or should Pathologists be a driving force for Personalized Medicine? This talk will begin with a brief overview of the healthcare landscape from a "genomic" perspective. It will define and explain some of the key components of personalized healthcare. Personalized Medicine will be discussed in the context of the US Healthcare System. Provocative ideas about the role of pathologists will be discussed in the context of

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the current debates and implications for the future practice of medicine.

Archived webinar presentation slides available

C) Doctors' Mistakes in Genetic Test Orders Is Warning Signal to Pathologists and Clinical Laboratories

McLeod P. Doctors' Mistakes in Genetic Test Orders Is Warning Signal to Pathologists and Clinical Laboratories. *Dark Daily.* October 29, 2012.

Summary: Almost one-third of medical laboratory test orders for complex gene tests contained mistakes in handling by ordering clinicians. This finding comes from a study by ARUP Laboratories, Inc.. The finding is an early warning flag for pathologists and clinical laboratory professionals that a gap exists between the availability of genetic tests and clinician knowledge of how and when to use them and how to interpret the results.

Free full text available from *Dark Daily*

D) AMP Test Directory

Carter AB, editor**.** AMP Test Directory [Internet]. Bethesda, MD: Association for Molecular Pathology, Bethesda; 2003-2012. Available from: http://www.amptestdirectory.org/index.cfm.

Summary: Provided as a service to its members and the public, the AMP Test Directory contains information concerning laboratory research or clinical tests provided by AMP members and their organizations. Information is submitted voluntarily and can be helpful in identifying laboratories that might serve a given need.

Full text available from AMP Test Directory

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Quick Reference Table: Selected Tests by Tumor Type

Tumor Type	Gene/Loci	Somatic Alteration	Clinical Use	References
Colorectal Adenocarcinoma				
	KRAS codons 12, 13, 61	Mutation	Lack of response to EGFR monoclonal antibodies	1-5, 41
	NRAS codons 12, 13, 61	Mutation	Lack of response to EGFR monoclonal antibodies	2,5
	BRAF	p.V600E mutation	Lack of response to EGFR monoclonal antibodies, MSI stratification, prognostic factor	2,4,6-8, 41
	MLH1	Promoter methylation	Indicates sporadic MSI Tumor	6, 41
Lung Adenocarcinoma				
	EGFR exons 18-21	Mutation	Response to EGFR inhibitors	9-12, 40
	EGFR	p.T790M mutation	Resistance to EGFR inhibitors	13-16, 40
	KRAS codons 12,13,61	Mutation	Exclusion of EGFR mutation	15,17,18, 40
	ALK	Rearrangement	Response to TKI	15,17,19, 40

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	ROS1	Rearrangement	Response to TKI	[17,20]
	MET	Amplification	Resistance to EGFR inhibitors	[17,21, 40]
Breast Carcinoma				
	HER2/ ERBB2	*Amplification*	*Response to HER2 monoclonal antibodies*	[22, 42]
Gastric Adenocarcinoma				
	HER2/ ERBB2	Amplification	Response to HER2 monoclonal antibodies	[23]
Thyroid Carcinoma				
Papillary Thyroid Carcinoma	*BRAF*	p.V600E mutation	Pre-operative FNA diagnosis and prognosis	[24]
	NRAS, HRAS, KRAS	Mutation	Pre-operative FNA diagnosis	[24]
	RET-PTC	Rearrangement	Pre-operative FNA diagnosis	[24]
Follicular Thyroid Carcinoma	*NRAS, HRAS, KRAS*	Mutation	Pre-operative FNA diagnosis	[24]
	PAX8-PPAR	Rearrangement	Pre-operative FNA diagnosis	[24]
Melanoma				
Cutaneous & Mucosal	*BRAF* codon 600	Mutation	Response to BRAF inhibitors	[25-27]
	KIT	Mutation	Response to TKI	[28]
Uveal	*GNAQ* or *GNA11*	Mutation	Diagnostic	[29]
	Chromosome 3	Loss (monosomy)	Unfavorable prognosis	[30]

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GIST				
	KIT	Mutation	Response to TKI	[31]
	PDGFRA	Mutation	Response to TKI	[31]
CNS Neoplasms				
Glioma	*MGMT*	Promoter methylation	Favorable response to alkylating agents	[32]
	IDH1 and *IDH2*	Mutation	Distinguishes reactive gliosis from glioma, favorable prognosis	[33,34]
Oligodendroglioma	Chromosome 1p and 19q	Co-deletion	Favorable prognosis and response to therapy	[35,36]
Pilocytic Astrocytoma	*BRAF*	Duplication/fusion	Diagnostic	[33]
Pleomorphic Xanthoastrocytoma	*BRAF*	p.V600E mutation	Diagnostic	[37]
Cholangiocarcinoma/Pancreatic Carcinoma				
	KRAS codons 12, 13, 61	Mutation	Pre-operative bile duct brushing diagnosis	[38]
Oropharyngeal Squamous Cell Carcinoma				
	HR HPV-related	Positive detection	Favorable response to chemoradiation therapy	[39]

MSI = Microsatellite Instability; TKI = Tyrosine-Kinase Inhibitors; HR HPV= High Risk Human Papillomavirus
This table is meant to be a list of selected tests and is not comprehensive.

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1.3 Opportunities for Pathologists in Personalized Medicine

A) Molecular Pathology in Contemporary Diagnostic Pathology Laboratory: An Opinion for the Active Role of Surgical Pathologists

Lauwers GY, Black-Schaffer S, Salto-Tellez M. Molecular pathology in contemporary diagnostic pathology laboratory: an opinion for the active role of surgical pathologists. *Am J Surg Pathol.* 2010 Jan; 34(1):115–117.

Summary: It may come to the surprise of many practicing surgical pathologists that as far back as 1966, someone who was certainly a traditional anatomic pathologist emphasized the role of molecular findings in pathology. A plea for a balanced approach was made, something with which few would argue; yet divisions exist, if we are honest, between those with unrealistic view of molecular pathology as potentially replacing surgical pathology and those with the opposite attitude. The opinion that follows reflects the experiences of diagnostic pathologists who wish to explore a middle ground, arguing for the active participation of surgical pathologists in the application of molecular techniques.

Full text available from *Lippincott Williams & Wilkins* (USD 59.00)
PMID: 19809276

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B) New Approaches to Molecular Diagnosis

Korf BR, Rehm HL. New approaches to molecular diagnosis. *JAMA.* 2013 Apr 10; 309(14): 1511-1521.

Summary: Advances in understanding the molecular basis of rare and common disorders, as well as in the technology of DNA analysis, are rapidly changing the landscape of molecular genetic and genomic testing. High-resolution molecular cytogenetic analysis can now detect deletions or duplications of DNA of a few hundred thousand nucleotides, well below the resolution of the light microscope. Diagnostic testing for "single-gene" disorders can be done by targeted analysis for specific mutations, by sequencing a specific gene to scan for mutations, or by analyzing multiple genes in which mutation may lead to a similar phenotype. The advent of massively parallel next-generation sequencing facilitates the analysis of multiple genes and now is being used to sequence the coding regions of the genome (the exome) for clinical testing. Exome sequencing requires bioinformatic analysis of the thousands of variants that are identified to find one that is contributing to the pathology; there is also a possibility of incidental identification of other medically significant variants, which may complicate genetic counseling. DNA testing can also be used to identify variants that influence drug metabolism or interaction of a drug with its cellular target, allowing customization of choice of drug and dosage. Exome and genome sequencing are being applied to identify specific gene changes in cancer cells to guide therapy, to identify inherited cancer risk, and to estimate prognosis. Genomic testing may be used to identify risk factors for common disorders, although the clinical utility of such testing is unclear. Genetic and genomic tests may raise new ethical, legal, and social issues, some of which may be addressed by existing genetic nondiscrimination legislation, but which also must be addressed in the course of genetic counseling. The purpose of this article is to assist physicians in recognizing where new approaches to genetic and genomic testing may be applied clinically and in being aware of the principles of interpretation of test results.

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Full text available from *Journal of the American Medical Association* (subscription required)
PMID: 23571590

C) **A National Agenda for the Future of Pathology in Personalized Medicine: Report of the Proceedings of a Meeting at the Banbury Conference Center on Genome-Era Pathology, Precision Diagnostics, and Preemptive Care: A Stakeholder Summit**
Tonellato PJ, Crawford JM, Boguski MS, Saffitz JE. A national agenda for the future of pathology in personalized medicine: report of the proceedings of a meeting at the Banbury Conference Center on genome-era pathology, precision diagnostics, and preemptive care: a stakeholder summit. *Am J Clin Pathol.* 2011 May; 135(5): 668-672.

Summary: In October 2010, representatives and thought leaders from major national pathology organizations and a diverse group of other stakeholders gathered at the Banbury Conference Center, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, to examine opportunities and challenges facing the discipline of pathology and its future role in the rapidly developing field of personalized medicine. A major focus of the meeting was assessment of the potential impact of next-generation sequencing (NGS) and whole-genome analysis (WGA) in medicine and, specifically, in clinical laboratory practice. (We define WGA as the sequencing of DNA and the alignment, variation calling, quality estimation, and annotation of one entire human genome.) The clearly articulated goal of the pathologists in attendance was to develop a national strategy to ensure that the performance, interpretation, and regulation of genome-based clinical testing come directly under the purview of pathologists and their national organizations.

Free full text available from *American Journal of Clinical Pathology*
PMID: 21502420

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1.4 Setting up a Molecular Lab

A) Tissue Handling and Specimen Preparation in Surgical Pathology: Issues Concerning the Recovery of Nucleic Acids from Formalin-Fixed, Paraffin-Embedded Tissue

Hewitt SM, Lewis FA, Cao Y, et al. Tissue handling and specimen preparation in surgical pathology: issues concerning the recovery of nucleic acids from formalin-fixed, paraffin-embedded tissue. *Arch Pathol Lab Med.* 2008 Dec; 132(12): 1929-1935.

Summary: Expression profiling by microarrays and real-time polymerase chain reaction-based assays is a powerful tool for classification and prognostication of disease; however, it remains a research tool, largely reliant on frozen tissue. Limiting the utility of expression profiling is the isolation of quality nucleic acids from formalin-fixed, paraffin-embedded tissue. The collection, handling, and processing of tissue directly impacts the biomolecules that can be recovered from it. High-quality nucleic acids can be obtained from formalin-fixed, paraffin-embedded tissue, but greater attention to all steps in the process of tissue handling and preparation is required. OBJECTIVE: To summarize the current state-of-the-art of preanalytic factors in tissue handling and processing as they impact the quality of RNA obtainable from formalin-fixed, paraffin-embedded tissue. The goals are to provide recommendations that will improve RNA quality for expression profiling from formalin-fixed, paraffin-embedded tissue and highlight areas for additional research. Tissue is an analyte and it must be handled in a standardized fashion to provide consistent results. DATA SOURCES: The literature was reviewed. Consultation with industry and academic leaders in the use of RNA for expression profiling was obtained to identify areas for additional research. CONCLUSIONS: Development of RNA-based assays from formalin-fixed, paraffin-embedded tissue is feasible. Greater attention to tissue handling and processing is essential to improve the quality of biospecimens for the development of robust RNA-based assays. Standardization of procedures and vigorous testing of alternative protocols are required to ensure that these assays function as designed.

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PMID: 19061293
NOTE: Also cited in Section 3.2.3

1.5 Industry Trends in Molecular Medicine

A) The Case for Personalized Medicine
Personalized Medicine Coalition. The Case for Personalized Medicine. 2011.

Summary: The Personalized Medicine Coalition (PMC), representing a broad spectrum of academic, industrial, patient, provider, and payer communities, seeks to advance the understanding and adoption of personalized medicine concepts and products for the benefit of patients. As part of its mission, the PMC publishes The Case for Personalized Medicine. Since the first edition was published three years ago, the number of prominent examples of personalized medicine treatments and diagnostics has increased from 13 products (69 percent of which were for cancer) to 37 products (56 percent of which were for cancer). The PMC is now pleased to release the second edition of The Case for Personalized Medicine. This report details how personalized medicine plays an increasingly integral role in delivering high-quality, cost-effective health care and presents evidence that personalized medicine will continue to grow in importance as scientific breakthroughs are translated into a new generation of targeted therapeutics. The report also surveys the opportunities and challenges that might affect the pace of adoption, and features comments from industry and government on the potential of personalized medicine and its place in the future of health care. This report is underwritten in part by the Ernst & Young Global Biotechnology Center.

Free full text available from *Journal of Diabetes Science and Technology*
PMID: 20144313

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1.5.1 International Standards in Molecular Medicine

A) Establishing Molecular Testing in Clinical Laboratory Environments; Approved Guideline

CLSI. Establishing Molecular Testing in Clinical Laboratory Environments; Approved Guideline. CLSI document MM19-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.

Summary: This guideline provides comprehensive guidance for planning and implementation of molecular diagnostic testing, including strategic planning, regulatory requirements, implementation, quality management, and special considerations for the subspecialties of molecular genetics, infectious diseases, oncology, and pharmacogenetics.

Full text available from American National Standards Institute (USD 120.00)

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Section 2 Insights from Adopters

Opinions expressed in this section are the authors' own and do not necessarily reflect an endorsement by CAP of any organizations, equipment, reagents, materials or services used by participating laboratories.

Alexis B. Carter, MD, FCAP, ASCP

2.1 Insights from Alexis Byrne Carter, MD, FCAP, ASCP

Alexis Carter, MD, FCAP is the Director of Pathology Informatics for the Department of Pathology and Laboratory Medicine at Emory University. A member of the College of American Pathologists' (CAP) Personalized Healthcare Committee and Digital Pathology Working Group, Dr. Carter is Special Interest Group (IPaLM SIG) of the International Health Terminology Standards Development Organisation (IHTSDO) which is the governing body for SNOMED CT Terminology. She serves on the Publication Committee of the Association for Molecular Pathology (AMP) and also is the editor of the AMP online test directory. She is a section editor for informatics for *Archives of Pathology and Laboratory Medicine*, is on the editorial board of the *Journal of Pathology Informatics* and is the Chair of the Training and Education Committee of the Association for Pathology Informatics.

Dr. Carter received her Bachelor of Science in Biochemistry from the University of Georgia in Athens, Georgia; her medical degree from Medical College of Georgia in Augusta, Georgia; and completed her residency training in Anatomic and Clinical Pathology at East Carolina University and Pitt County Memorial Hospital in Greenville, North Carolina. Following residency, she completed a fellowship in Molecular Genetic Pathology at the University of Pittsburgh Medical Center followed by an additional year of research in Molecular Diagnostics and Informatics at the same institution. She is board-certified in Anatomic, Clinical and Molecular Genetic Pathology. Her research interests include development of robust clinical information systems for molecular and

genomics laboratories, telepathology for patient care, communication of complex laboratory results to providers and patients and electronic identification systems for patients and specimens.

Dr. Carter's insights for the next adopters (September 2012):

1	**Get up to speed on molecular testing**	Have someone in your practice get up to speed on molecular testing enough to answer basic questions from non-pathologists.
2	**Find local genetic counselors**	Find out who your local genetic counselors and geneticists are and how to refer patients and clinical providers to them.
3	**Tour a molecular pathology lab**	If there is a molecular pathology laboratory in your area, ask to take a tour through the lab and to meet the pathologists and laboratorians who run it. Molecular pathologists and laboratorians LOVE giving tours to other interested pathologists.
4	**Have a plan**	Have a plan to deal with requests for molecular tests when they hit the door.
5	**Create a test utilization committee**	Consider having a test utilization committee to review molecular and other tests on a proactive basis to ensure that your patients are getting the best and most efficient care possible.
6	**Use tests with medical literature**	Be wary of any test which does not have any medical literature to support it.

Dr. Carter's suggested articles and resources

A) Clinical Requests for Molecular Tests: The 3-Step Evidence Check

Carter AB. Clinical requests for molecular tests: the 3-step evidence check [published online ahead of print April 5, 2012]. *Arch Pathol Lab Med.* 2012. http://dx.doi.org/10.5858/arpa.2011-0691-SA

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Summary: Laboratory tests performed by molecular methods are increasing in volume and complexity at an unprecedented rate. Molecular tests have a broad set of applications, and most recently have been advocated as the mechanism by which providers can further tailor treatments to the individual patient. As the momentum behind molecular testing continues to increase, pathology practices may find themselves unprepared for the new wave of molecular medicine. This special article has been developed in an effort to provide pathologists who have limited molecular training with a simple and quick algorithm for determining whether a requested molecular test is appropriate for a patient. Additional recommendations for a more intensive and proactive review and management of molecular requests also are included. The principles discussed can easily be applied to requests for any test, including those not using molecular methods, which would be sent to an outside reference laboratory. This special article was developed from a Webinar for the College of American Pathologists targeting education for pathologists about the transformation of pathology practice in the new molecular and digital age.

Free full text available from the CAP's *Archives*
PMID: 22480222
NOTE: Also cited in Section 1.2

B) Public Policy Recommendations for Oversight of Molecular Laboratory Tests

Gulley ML. Public Policy Recommendations for Oversight of Molecular Laboratory Tests.
N C Med J. 2007;68(2):109-111.

Summary: Laboratory tests have long been used to help diagnose and classify disease. Increasingly, these assays are used to predict disease in healthy individuals or to predict outcomes in response to a specific therapy (See Table 1). The subspecialty of molecular genetic pathology (MGP) has recently emerged to promote and recognize physician expertise in DNA- and RNA-based testing. In fact, the University of North Carolina at Chapel Hill has the nation's first accredited MGP fellowship training program to graduate a physician who subsequently became board-certified.

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Free full text available from *North Carolina Medical Journal*
PMID: 17566555

C) Clinical Laboratory Reports in Molecular Pathology
Gulley ML, Braziel RM, Halling KC, et al. Clinical Laboratory Reports in Molecular Pathology. *Arch Pathol Lab Med.* 2007 June; 131(6):852-863.

Summary: Molecular pathology is a rapidly growing area of laboratory medicine in which DNA and RNA are analyzed. The recent introduction of array technology has added another layer of complexity involving massive parallel analysis of multiple genes, transcripts, or proteins. Objective.—As molecular technologies are increasingly implemented in clinical settings, it is important to bring uniformity to the way that test results are reported. Data Sources.—The College of American Pathologists Molecular Pathology Resource Committee members summarize elements that are already common to virtually all molecular pathology reports, as set forth in the College of American Pathologists checklists used in the laboratory accreditation process. Consensus recommendations are proposed to improve report format and content, and areas of controversy are discussed. Resources are cited that promote use of proper gene nomenclature and that describe methods for reporting mutations, translocations, microsatellite instability, and other genetic alterations related to inherited disease, cancer, identity testing, microbiology, and pharmacogenetics. Conclusions.—These resources and recommendations provide a framework for composing patient reports to convey molecular test results and their clinical significance to members of the health care team.

Free full text available from the CAP's *Archives*
PMID: 17550311

D) Nomenclature for the Description of Sequence Variations
den Dunnen J. Nomenclature for the description of sequence variations. Genetic variations standards in reporting. 2007. Available at: http://www.hgvs.org/mutnomen/. Accessed August 13, 2007.

Summary: A nomenclature system has recently been suggested for the description of changes (mutations and polymorphisms) in DNA

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and protein sequences. These nomenclature recommendations have now been largely accepted. However, current rules do not yet cover all types of mutations, nor do they cover more complex mutations. This document lists the existing recommendations and summarizes suggestions for the description of additional, more complex changes. Another version of this paper has been published in Hum Mut 15:7-12, 2000.

Full text available from *Human Genetics* (USD 39.95)
PMID: 11479744

E) Mutation Nomenclature Extensions and Suggestions to Describe Complex Mutations: A Discussion
den Dunnen JT, Antonarakis SE. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. *Hum Mutat*. 2000;15(1):7-12.

Summary: Consistent gene mutation nomenclature is essential for efficient and accurate reporting, testing, and curation of the growing number of disease mutations and useful polymorphisms being discovered in the human genome. While a codified mutation nomenclature system for simple DNA lesions has now been adopted broadly by the medical genetics community, it is inherently difficult to represent complex mutations in a unified manner. In this article, suggestions are presented for reporting just such complex mutations.

Free full text available from *Human Mutation*
PMID: 10612815

F) Guidelines for Human Gene Nomenclature
Wain HM, Bruford EA, Lovering RC, Lush MJ, Wright MW, Povey S. Guidelines for human gene nomenclature. *Genomics.* Apr 2002;79(4):464-470.

Summary: Guidelines for human gene nomenclature were first published in 1979 [1], when the Human Gene Nomenclature Committee was first given the authority to approve and implement human gene names and symbols. Updates of these guidelines were published in 1987 [2], 1995 [3], and 1997 [4]. With the recent publications of the complete human genome sequence there is an estimated total of 26,000–40,000 genes, as suggested by the

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International Human Genome Sequencing Consortium [5] and Venter et al. [6]. Thus, the guidelines http://www.gene.ucl.ac.uk/nomenclature/guidelines.html have been updated to accommodate their application to this wealth of information, although gene symbols are still only assigned when required for communication. These updates were derived with input from the HUGO Gene Nomenclature Committee (HGNC) International Advisory Committee and attendees of the ASHG01NW Gene Nomenclature Workshop. All approved human gene symbols can be found in the Genew database [7].

Full text available from *Genomics* (USD 31.50)
PMID: 11944974

Samuel K. Caughron, MD, FCAP

2.2 Insights from Samuel K. Caughron, MD, FCAP

Samuel K. Caughron, MD, FCAP, is a member of the MAWD Pathology Group in Kansas City. In 2009 Dr. Caughron joined MAWD Pathology, a 12 pathologist group, to establish a community based molecular pathology lab. He currently is Director of the MAWD Molecular Lab in addition to practicing routine anatomic and clinical pathology at a 450 bed community hospital. Dr. Caughron had previously practiced in Billings, Montana where he helped establish a molecular lab for a 7 member pathology group. Dr. Caughron serves as vice-chair of the College of American Pathologists' (CAP) Personalized Health Care Committee, is a member the Transformation Program Office Steering Committee and a representative to the CAP House of Delegates.

Dr. Caughron received his medical degree from Creighton University School of Medicine in Omaha, Nebraska; he also completed his residency training in Anatomic and Clinical Pathology there. After residency, he completed a fellowship in Molecular Genetic Pathology at Vanderbilt University Medical Center in Nashville, Tennessee. He is board certified in Anatomic and Clinical Pathology, as well as Molecular Genetic Pathology.

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Dr. Caughron's insights for the next adopters (September 2012):

1	**Move beyond your comfort zone**	Molecular pathology is a new area for many pathologists, especially those who have been in practice since before it was a part of pathology training. But even for those who are trained in molecular pathology, the field is moving so quickly that yesterday's conclusions may not be true today. Adoption of molecular testing requires practicing in an area where things can change rapidly, where the basis for decisions will not be as established - an area that is uncomfortable for many pathologists. Learn to accept and live with it to help patients the best you can.
2	**Find a way to stay current with new advances**	As molecular testing advances, there will be tremendous benefits possible for patient care. Today there are several examples like imatinib (Gleevec®) for CML, crizotinib (Xalkori®) for non-small cell lung cancer, and vemurafenib (Zelboraf®) for advanced melanoma. If you are going to get into molecular testing, even if you are not performing all of the testing, you have to stay current so that you know the possibilities for your patients.
3	**You don't have to "do it all" to adopt molecular pathology testing**	In fact, no lab does it all. Even the largest reference labs send specimens for some tests to other reference labs. Pick a focus for your lab based on your patient mix, requests from other specialties, or target areas for growth.

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4	**Take care of your own patients**	Don't give up that job to another doctor or lab. This does not mean you have to perform all the testing for your patients. You cannot. But if there are tests being sent out to other labs, find out what the testing it being used for and what its real value is to the patient. If it does not make sense, add value and establish expertise by intervening. Do not allow yourself to be taken out of the loop for lab testing on your patients.
5	**Be a visible resource to your colleagues for molecular testing**	Take, or make, opportunities to present on topics relevant to your molecular lab. The CAP offers the Short Presentations on Emerging Concepts (SPECs) for exactly this purpose. After you have set up a test, you are a local experts on that test. Explain what you are doing, and help your colleagues make sense of the results. Pathology is not the only specialty struggling to keep pace with the advances. But we are the only specialty with the training to properly evaluate, implement, and interpret the testing.
6	**Take advantage of the national organizations offerings to support molecular pathology**	As mentioned above, the CAP offers the SPECs to jump start a presentation you are asked to give about some topics. The Association of Molecular Pathology has a tremendous number of resources available to help understand and implement molecular testing. And there are others. If you seek educational opportunities, you will find them in abundance.

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7	**Invest in understanding the coverage, coding and reimbursement for molecular testing**	This is a challenging, and rapidly changing area. However, the final success of your lab will probably depend on being able to make it financially viable. Coding is how you describe to payers what you did in your lab. Reimbursement is what you get paid. Coverage is whether you get paid. All of these crucial ingredients have to be in the proper mix to achieve success. Get to know them well.
8	**Understand the big picture and true value of the testing you offer**	Molecular testing is really in its infancy or early childhood. There is a lot of growth yet to happen. Adopting molecular testing today will yield fruit down the road, but only if it fits with the delivery of healthcare in your area. Bringing valuable testing online may not immediately generate a revenue stream, but may be compelling for its cost savings in other areas of care. Find the opportunity and real value. You may have to give it time. You may have to sell it to colleagues, to payers, to administration. But it it there or will come.
9	**Work with good people**	My father once told me, "You can never go wrong hiring good people." The success of your molecular efforts will depend entirely on the people who are involved, from the medical technologist, to the billing department, to the support staff. Find the best possible people you can for each job and treat them well. You will never regret it.

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10	**Think like a doctor**	As pathologists, we can get caught up in our analyses and lose sight of what the information we provide really means to the care and life of the patient. Molecular pathology is an opportunity for pathologists to claim a new and vital role in helping to take care of patients, by providing critical new kinds of information. When adopting or performing a test, keep asking: What is the clinical value to a test being performed? How will it impact treatment?

Dr. Caughron's suggested articles and resources:

A) **PubMed.com and Google.com**
While not traditional resources, online search capability is essential to staying current. I could not do my job without rapid access to information. Take a few minutes to become familiar with optimizing searching on PubMed and Google. It will yield tremendous returns.

B) **CAP Accreditation Checklist for Molecular Labs**

C) **Oncology journals**

Frederick L. Kiechle, MD, PhD, FCAP

2.3 Insights from Frederick L. Kiechle, MD, PhD, FCAP

Frederick L. Kiechle, MD, PhD, FCAP, is the Medical Director of Clinical Pathology for Memorial Healthcare System in Hollywood, Florida. He is also the Vice-President of the Department of Pathology at Memorial Regional Hospital for Pathology Consultants at South Broward, LLP. Dr. Kiechle is board certified in Clinical and Anatomic Pathology and he has been practicing Clinical Pathology for over 30 years. Dr. Kiechle serves as an advisor for the College of American Pathologists' (CAP) Publication Committee and is also a member of the CAP Chemistry Resource Chemistry.

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Dr. Kiechle has initiated two molecular diagnostics laboratories: William Beaumont Hospital, Royal Oak, Michigan in 1991 and Memorial Healthcare System, Hollywood, Florida in 2006. The Michigan lab offered 33 billable procedures by August 2005 in human genetics, hematopathology and infectious diseases. The Florida lab offers 21 billable procedures currently. All of these molecular diagnostic assays are in the infectious diseases area. This current narrow focus addresses the clinical needs expressed by the infectious disease practitioners who need a turnaround time of less than 24 hours for many of the microbe and/or viral assays they order for their patients. Delay may result in increased disease severity with resultant increased morbidity and/or mortality.

Dr. Kiechle's Insights for the Next Wave of Adopters (August 2012):

1	**Identify medical subspecialties who would benefit or already order molecular diagnostic assays.**	This information will help define the molecular diagnostic assay needs at your institution and/or for your outreach clients.
2	**Develop a business plan based on the information in 1B.**	The business plan will define the expenses related to equipment, space, personnel, cost per test and potential reimbursement needed to assess profitability of the proposed molecular diagnostics lab.
3	**Identify personnel including medical director, technical director and lab personnel.**	The complexity of the test menu may require input from more than one medical director (pathologist, medical geneticist, infectious disease, etc) as well as more than one technical director. Start small and grow incrementally.
4	**Design lab space for incremental growth based on 5-year business plan.**	The most frequent error is to start with a clean room for nucleic acid extraction and master mix preparation and space for associated analyzers that will not accommodate growth in the future years.

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5	**Organize the introduction of new assays to utilize (maximize) one analyzer at a time.**	The manufacturers of random-access molecular devices either product very large or very small devices. Design adequate space for current choices. It is very difficult to guess the future landscape in the next 5 years.
6	**Become familiar with financial issues like the effect of patents on molecular diagnostics, cost justification for bringing a send out test in-house, hospital cost avoidance model used for MRSA, enterovirus, C. diff., and Group B Strep, and revenue cycle.**	Sometimes the justification for the introduction of a new assay will use some of these financial elements.
7	**Consider the positive impact on Outreach (inreach) program will have on the molecular lab.**	Outreach (inreach) programs bring in additional test volume. Marketing the molecular lab will increase utilization, reduce unit cost per test and increase margin.
8	**Review molecular lab volume, expenses, reimbursement and margin every month.**	Over 95% of molecular assay have a positive margin currently. However, changes in reimbursement policies (stacking codes to no stacking codes) may alter this financial picture.

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Dr. Kiechle's suggested articles and resources:

A) Real-time PCR in Clinical Microbiology: Application for Routine Laboratory Testing

Espy MJ, Uhl JR, Sloan LM, et al. Real-time PCR in clinical microbiology: Applications for routine laboratory testing. *Clin Microbiol Rev* 2006; 19:165-256.

Summary: Real-time PCR has revolutionized the way clinical microbiology laboratories diagnose many human microbial infections. This testing method combines PCR chemistry with fluorescent probe detection of amplified product in the same reaction vessel. In general, both PCR and amplified product detection are completed in an hour or less, which is considerably faster than conventional PCR detection methods. Real-time PCR assays provide sensitivity and specificity equivalent to that of conventional PCR combined with Southern blot analysis, and since amplification and detection steps are performed in the same closed vessel, the risk of releasing amplified nucleic acids into the environment is negligible. The combination of excellent sensitivity and specificity, low contamination risk, and speed has made real-time PCR technology an appealing alternative to culture- or immunoassay-based testing methods for diagnosing many infectious diseases. This review focuses on the application of real-time PCR in the clinical microbiology laboratory.

Free full text available from PubMed
PMID: 16418529
NOTE: Also cited in Section 5.2

B) Molecular Detection and Surveillance of Healthcare-Associated Infectious

Rao A, Foder B, Hocker K. Molecular detection and surveillance of healthcare-associated infectious. In: Molecular Diagnostics: Techniques and applications for the clinical laboratory. (Eds: Grody WW, Nakamura RM, Strom CM, Kiechle FL). Academic Press, Inc: Boston, MA, 2010: pp 327-346.

Book available for purchase from Amazon

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C) Outreach Implementation Requirements: A Case Study

Kiechle FL, Skrisson JE. Outreach implementation requirements: a case study. In: Clinical Laboratory Management. (Ed: Garcia L.). American Society for Microbiology: Washington, DC. 2004: 654-671.

Summary: Illustrates the positive effect the increased test volume from successful outreach program had on the growth of a hospital-based molecular diagnostics lab in Michigan.

Book available for purchase from Amazon

D) Point-of-Care Testing and Molecular Diagnostics: Miniaturization Required

Kiechle FL, Holland CA. Point-of-Care testing and molecular diagnostics: Miniaturization required. *Clin Lab Med* 2009; 29:555-560.

Summary: Turnaround time for molecular diagnostic tests is critical in detecting infectious agents, in determining a patient's ability to metabolize a drug or drug class, and in detecting minimal residual disease. These applications would benefit from the development of a point-of-care device for nucleic acid extraction, amplification, and detection. The ideal device would have a low cost per test, use a disposable unit use device for all steps in the assay, be portable, and provide a result that requires no interpretation. The creation of such a device requires miniaturization of current technologies and the use of microfluidics, microarrays, and small-diameter capillary tubes to reduce reagent volumes and simplify heat conduction by convection during nucleic acid amplification. This ideal device may be available in 3 to 5 years and will revolutionize and expand the global availability of molecular diagnostic assays.

Full text available from *Clinics in Laboratory Medicine* (USD 31.50)
PMID: 19840687

E) Molecular Pathology and Infectious Diseases

Kiechle FL. Molecular pathology and infectious diseases. In: Molecular Diagnostics: Techniques and Applications for the Clinical Laboratory. (Eds: Grody WW, Nakamura RM, Strom CM, Kiechle FL). Academic Press, Inc.: Boston, MA. 2010: 99-106.

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Book available for purchase from Amazon

David G. Hicks, MD, FCAP

2.4 Insights from David G. Hicks, MD, FCAP

David G. Hicks, MD, FCAP, is currently the director of Surgical Pathology at the University of Rochester Medical Center. Dr. Hicks earned his medical degree from the University of Rochester School of Medicine and Dentistry. Dr. Hicks' current research interests focus on the molecular profiling of clinical samples from breast cancer patients, with the goal of identifying new biomarkers to help better understand the prognosis and guide the therapeutic management of this disease. Dr. Hicks participated in the ASCO/CAP ER/PgR Guideline panel and is currently *serving as Co-Chair of the ASCO-CAP HER2 Testing in Breast Cancer Committee.*

Dr. Hicks has co-authored over 140 peer reviewed articles that have appeared in a variety of journals, including Clinical Cancer Research, The American Journal of Pathology, Cancer and the American Journal of Surgical Pathology. He also serves on the editorial boards of the Archives of Pathology and Laboratory Medicine, Biotechnic and Histochemistry and Applied Immunohistochemistry and Molecular Morphology. As part of the working group for the CAP BPFT AP^3 Program, Dr. Hicks has contributed significantly to the overall direction and development of the BPFT curriculum and assessments.

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Section 2

Dr. Hicks' Insights for the Next Wave of Adopters

1	**Stay informed on treatment information related to molecular analysis and breast cancer care**	Seek out and review new literature related to the molecular analysis of clinical samples and how these studies help inform treatment decisions. In doing so, you will be better equipped to discuss the benefits and limitations of molecular testing in the clinical setting. There are excellent articles and educational offerings from the College as well as other pathology organizations on this topic that can help make the pathologist a 'clinical consultant on the biology of disease' for other members of the multidisciplinary care team.
2	**Help ensure the biologic quality of tissue samples for biomarker studies**	Provide leadership and guide your institution's efforts to review procedures related to tissue collection and fixation with a movement towards standardizing pre-analytic variables. As molecular analysis of tissue samples becomes increasingly applicable to clinical care, the accuracy, reliability and relevance of this approach, and the tissue requirements for testing need to be addressed. There is a growing awareness that variable tissue handling and prolonged cold ischemic times can adversely affect the quality of breast tissue samples for ER, PR and HER2 testing and other molecular analysis.

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3	**Provide clear, concise, and comprehensive reports**	Develop a firm understanding of how your reports are used to make clinical decisions and make sure the information is presented in a clear, concise and understandable manner. Pathology reports need to be comprehensive in addressing all issues relevant to the patient's care as well as provide summary-level diagnostic information (including molecular test results) and recommendations that are patient/tumor specific.
4	**Become an active participant and consultant to the multidisciplinary care team**	Attend tumor boards and/or multidisciplinary treatment planning discussions. Be available as a diagnostic consultant and actively participate in these discussions and share information on how molecular testing can be used to inform clinical diagnosis and treatment decisions.
5	**Check that the biomarker results correlate with the clinical profile for each patient**	Review all results to verify that the molecular tests or profile are a reasonable fit with the clinical features for each breast cancer patient regardless of whether the testing (either single marker studies or multigene panels) is done in your laboratory or sent out. There is strong evidence in the literature that the histopathologic features of a breast cancer tumor correlate with its molecular profile. Low grade tumors with a low proliferative index typically will be ER/PR positive, HER2 negative and have a low recurrence score by the Oncotype DX test. High grade tumors with a high proliferative index are more likely to show low levels or an absence of ER expression, over-express HER2 and have a high recurrence score by the Oncotype DX test. If the molecular testing is dramatically different from the histopathologic features of the

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		tumor, the case should be thoroughly investigated by the pathologist in collaboration with medical oncology before decisions on adjuvant treatment are made.

Dr. Hicks' thoughts on molecular testing:

A) CAP Short Presentations in Emerging Concepts (SPECS): Emerging Concepts in Molecular Testing in Breast Cancer [PowerPoint slides]

College of American Pathologists. CAP Short Presentations in Emerging Concepts (SPECS): Emerging Concepts in Molecular Testing in Breast Cancer [PowerPoint slides]. Version 1.0. Northfield, IL: College of American Pathologists; 2013**.**

Access the slides here

B) Molecular Markers in Breast Cancer

Hicks DG. PHC Webinar. Molecular markers in breast cancer. [Webinar]. March 20, 2013. https://www1.gotomeeting.com/register/298012304. Accessed September 12, 2013.

Summary: Clinicians caring for breast cancer patients have known for years that this disease shows significant heterogeneity, encompassing a number of distinct biologic entities with widely varied pathologic features and clinical behavior. This poses a major challenge for clinician caring for patients as they try and determine the risk of recurrence and the most appropriate adjuvant treatment regimen. A number of different strategies have been used to try and stratify patients into subset of disease to better understand this diversity. Established clinical and pathologic features such as patient age, tumor size, nodal status, tumor grade, margin status, and ER, PR and HER2 status are currently used to determine a patient's likelihood for recurrence and subsequent treatment options. These important prognostic parameters are valuable and have been extensively validated in numerous clinical studies over many years of clinical practice. However, these types of risk estimates remain imprecise for many patients.

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Advances in biotechnology have brought us to the point where we can now analyze global genomic changes in clinical tissue samples from patients with breast cancer. These technical advances have shown great promise for the development of clinically relevant tumor classification, better assessment of prognosis and better prediction of response to therapy. In this webinar we will review some of these new approaches to profiling breast cancer patients as they rapid find their way into clinical practice.

Molecular subtypes of breast cancer are closely correlated with conventional histologic and immunophenotypes of breast cancer, which will be reviewed. With this technical revolution, the pathology community has an unprecedented opportunity to help interpret molecular information into the morphologic and clinical context for each patient and in doing so can become a valuable member of the multidisciplinary breast cancer treatment team and directly impact decision on appropriate treatments and patient outcomes.

Archived webinar available for free; presentation slides available

Dr. Hicks' suggested articles and resources:

A) The Role of the Indispensable Surgical Pathologist in Treatment Planning for Breast Cancer

Hicks DG, Kulkarni S, Hammond ME. The role of the indispensable surgical pathologist in treatment planning for breast cancer. *Arch Pathol Lab Med*. 2008 Aug;132(8):1226-7.

Summary: The treatment of breast cancer has become increasingly specialized with rapidly changing new therapies and therapeutic guidelines. The pace at which these changes have taken place has proven to be a challenge for the field of pathology but also represents an opportunity for knowledgeable surgical pathologists to assume a greater role in this increasingly complex and specialized environment of breast cancer care. This editorial written by two pathologists and a breast surgeon examines the evolving role of the surgical pathologist in treatment planning for breast cancer and discusses how the

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pathology community can become active consultants and participating members of the multidisciplinary care team.

Free full text available from the CAP's *Archives*
PMID: 18684020

B) Gene-expression Signatures in Breast Cancer
Sotiriou C, Pusztai L. Gene-expression signatures in breast cancer. *N Engl J Med.* 2009 Feb 19;360(8):790-800.

Summary: Gene-expression profiling with the use of DNA microarrays allows the measurement of thousands of gene transcripts in a single experiment. The application of this technology to clinical samples in numerous studies has confirmed that breast cancer is not a single disease but rather, a group of molecularly distinct neoplastic disorders. The results of these studies have revealed molecular profiles with prognostic and therapeutic implications that could influence clinical care. In addition to the intrinsic molecular classifications of breast cancer (luminal A, luminal B, HER2 enriched and basal-like carcinomas), there have been a number of different prognostic gene signatures that have been developed based on gene expression profiling data. Interestingly, there is little overlap between the different gene-signatures developed. Also, the different genes appear to map to critical pathways that underlie breast cancer biology, suggesting that each of these signatures track biologic characteristics that impact clinical behavior and outcomes in breast cancer. Genes associated with tumor differentiation and cell cycle drive the prognostic power of the intrinsic molecular classifications and other gene expression signatures. Each of these approaches is similar in their ability to stratify patients into high risk and low risk groups. Both morphology and the underlying gene expression patterns appear to track fundamental biologic characteristics which impact breast cancer.

Full text available from *New England Journal of Medicine*
(subscription required)
PMID: 19228622

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C) Molecular Pathology of Breast Cancer: What a Pathologist Needs to Know

Allison KH. Molecular pathology of breast cancer: what a pathologist needs to know. *Am J Clin Pathol.* 2012 Dec;138(6):770-80.

Summary: This article provides an excellent summary on the current state of knowledge of the molecular pathology of breast cancer, with recommendations for practicing pathologists in the new personalized health care era. A basic understanding of this knowledge is key to enabling pathologists to serve as clinical consultants. Currently, ER, PR and HER2 are single-marker molecular tests for adjuvant treatment planning and the accuracy of these results is heavily influenced by tissue handling, test methodology, validation, scoring and reporting. In addition, two commercially available multigene signatures – Oncotype DX and MammaPrint – are increasingly used to determine the treatment options for ER positive tumors. However, the results of these tests can be severely compromised by the presence of non-invasive tumor cells (inflammatory cells, in situ tumor cells and normal breast tissue). As the "diagnostic consultant", pathologists have a number of important roles including ensuring appropriate tissue handling, providing guidance on the benefit and limitation of molecular testing in different clinical settings, interpretation of results, and clinical correlation to help provide optimal patient care.

Full text available from *American Journal of Clinical Pathology* (USD 12.00)
PMID: 23161709

D) The Effect of Cold Ischemic Time on the Immunohistochemical Evaluation of Estrogen Receptor, Progesterone Receptor, and HER2 Expression in Invasive Breast Carcinoma

Yildiz-Aktas IZ, Dabbs DJ, Bhargava R. The effect of cold ischemic time on the immunohistochemical evaluation of estrogen receptor, progesterone receptor, and HER2 expression in invasive breast carcinoma. *Mod Pathol* 25:1098-1105.

Summary: In a study reported by Yildiz-Aktas et al, breast resection specimens were subjected to variable cold ischemic times within the refrigerator and at room temperature. These samples were

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processed and stained for ER, PR and HER2 and the results compared with the prior needle core biopsies from the same patient, which would have had a negligible cold ischemic time period before fixation. Significant reduction in IHC staining for hormone receptors and HER2 were not detected until 4 hours for refrigerated samples and after 2 hours for non-refrigerated samples. The authors concluded that the ASCO/CAP guideline of a cold ischemic time period of <1 hour is a prudent guideline to follow and that refrigeration of specimens that may encounter delays before the start of fixation may be warranted.

Free full text available from *Modern Pathology*
PMID: 22460807

E) Prediction of the Oncotype DX Recurrence Score: Use of Pathology-generated Equations Derived by Linear Regression Analysis

Klein ME, Dabbs DJ, Shuai Y, et al. Prediction of the Oncotype DX recurrence score: use of pathology-generated equations derived by linear regression analysis. *Mod Pathol*. 2013 May;26(5):658-64.

Summary: Oncotype DX is a commercial assay frequently used for making chemotherapy decisions in estrogen receptor (ER)-positive breast cancers. The result is reported as a recurrence score ranging from 0 to 100, divided into low-risk (<18), intermediate-risk (18-30), and high-risk (≥31) categories. Our pilot study showed that recurrence score can be predicted by an equation incorporating standard morphoimmunohistologic variables (referred to as original Magee equation). Using a data set of 817 cases, we formulated three additional equations (referred to as new Magee equations 1, 2, and 3) to predict the recurrence score category for an independent set of 255 cases. The concordance between the risk category of Oncotype DX and our equations was 54.3%, 55.8%, 59.4%, and 54.4% for original Magee equation, new Magee equations 1, 2, and 3, respectively. When the intermediate category was eliminated, the concordance increased to 96.9%, 100%, 98.6%, and 98.7% for original Magee equation, new Magee equations 1, 2, and 3, respectively. Even when the estimated recurrence score fell in the intermediate category with any of the equations, the actual recurrence score was either

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intermediate or low in more than 80% of the cases. Any of the four equations can be used to estimate the recurrence score depending on available data. If the estimated recurrence score is clearly high or low, the oncologists should not expect a dramatically different result from Oncotype DX, and the Oncotype DX test may not be needed. Conversely, an Oncotype DX result that is dramatically different from what is expected based on standard orphoimmunohistologic variables should be thoroughly investigated.

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Shannon J. McCall, MD, FCAP

2.5 Insights from Shannon J. McCall, MD, FCAP

Shannon McCall, MD, FCAP, is the Director of the College of American Pathologists (CAP)-Accredited Biospecimen Repository & Processing Core Facility at Duke University Medical Center and serves on the Biorepository Accreditation Program Committee. An Assistant Professor at Duke, Dr. McCall specializes in pathology of the gastrointestinal and hepatobiliary tracts. She serves as the Quality Assurance Officer for the Duke Anatomic Pathology Practice and is also a member of the College of American Pathologists' Quality Practices Committee.

Dr. McCall received Bachelor of Science degrees in Chemical Engineering and Biochemistry from North Carolina State University in Raleigh, North Carolina and her medical degree from Duke University in Durham, North Carolina. She also completed her residency training in Anatomic and Clinical Pathology as well as a one year fellowship in Gastrointestinal and Hepatic Pathology at Duke University. She is board-certified in Anatomic and Clinical Pathology. Her research interests include the developing automated quality assurance metric collection for anatomic pathologists for use in laboratory accreditation, credentialing, and new health system or government quality initiatives. She is also involved in the expansion of a novel business and governance model for biobanking at Duke as well as collaborative translational science in gastrointestinal oncology.

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Section 2

Dr. McCall's Insights for the Next Wave of Adopters

1	**Will Your Specimen Be "fit for purpose"?**	The catchphrase "fit for purpose" can be translated, "collecting the specimen with the required end assay(s) in mind." In prior years, anatomic pathology specimens were collected and processed for the end assay of paraffin embedding and histologic evaluation of a slide stained with hematoxylin and eosin. Numerous histochemical and, more recently, immunohistochemical and *in situ* hybridization staining protocols evolved out of this background, and have been optimized for use with formalin-fixed, paraffin-embedded (FFPE) material. For molecular assays dependent on specimens other than FFPE, however, there is only one chance to optimize collection. While many DNA-based assays demonstrate solid performance using FFPE material, in-situ hybridization (ISH) assays often perform better using touch preparations of tumor cells. RNA-based assays (such as QT-PCR) perform best with frozen tissue, however, almost all have been optimized for FFPE. The current CAP-American Society of Clinical Oncology (ASCO) recommendation for the cold ischemia time, the period between removal of the biospecimen from the patient and its dissection (if necessary) and preservation, is less than 60 minutes. The cold ischemia time should be put into the pathology report.

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2	**Know that you're already practicing "molecular pathology."**	Acknowledge that when you are utilizing immunohistochemistry and/or *in situ* hybridization to interrogate your tumor samples for protein or nucleic acid expression, respectively, you are already practicing molecular pathology. It's a short step from there to performing quantitative assessments of biomarker expression (such as Ki-67, estrogen receptor (ER), progesterone receptor (PgR) and HER2 testing). Expanding your practice to include interrogating your tumor samples for KRAS mutations or microsatellite instability simply builds on and adds value to your current practice.
3	**Add a few more data points to your specimen processing protocols to ensure compliance with routine molecular oncology testing.**	Guidelines for molecular biomarker testing in ER/PgR, published jointly by CAP-ASCO in 2010, and to be aligned with their HER2 guidelines in late 2013, intended to reduce preanalytic variability specify minimum and maximum fixation times for breast specimens in 10% neutral phosphate buffered formalin (6-72 hours). The time into formalin and estimated "time out of formalin", or the total time the biospecimen was in formalin, should be documented in the pathology report to assure downstream compliance with these best practices. When "unusual" or "unexpected" results occur, knowing the cold ischemia time and the total time in formalin will be very helpful in troubleshooting the potential causes for the discrepancy.

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4	**Consider adding a few more data points to your specimen processing protocols to maximize the downstream value of your archival biospecimens as retrospective research samples.**	Today's retrospective research samples can quickly become tomorrow's validation of the next diagnostic assay. This is the nature of translational research. Expanded preanalytic variables, if not documented at the time of specimen processing, may be very difficult to document at a later time. Documentation of selected preanalytic variables may substantially increase the downstream value of your tissue samples in future clinical or research assays. As an example, prior tumor treatment with chemotherapy and/or radiation should be documented in the pathology report.
5	**Don't throw the baby out with the bathwater.**	Once you've considered the value of your tissues for downstream clinical molecular testing and research, as well as for potential future patient care, you may be tempted make major changes in your processing protocols to accommodate the widest variety of downstream assays. Since the majority of anatomic pathology practices (histology, histochemistry, and immunohistochemistry) have been validated in FFPE however, switching or altering your fixation protocols to include substances like Allprotect or RNAlater could seriously impair your ability to interpret routine IHC slides.
6	**Once molecular tests have been ordered, refresh yourself on the basics so that you know the best sample to provide.**	Today's molecular assays evaluate many different areas. Will the test you need involve sequencing tumor DNA for a mutation, comparing expansion of microsatellites in tumor and normal, or using fluorescent probes to identify a chromosomal translocation in tumor? There is a difference between submitting a

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		representative tumor block for testing using a notation in the surgical pathology report, which is a good practice, and reviewing the case yourself to select a block specifically for the downstream assay. Traditional DNA sequencing assays could require 30% tumor nuclei and a minimum size. Microsatellite instability assays require samples of both tumor and normal tissue. Consult the molecular lab for specific assay requirements. Consider submitting a separate block of viable tumor during gross evaluation to facilitate downstream molecular testing, if your consenting process and workflow permit. You may also consider placing multiple pieces of both normal and malignant tissue in the same block. This multiple piece technique is useful, because a normal control is simultaneously processed with the test tissue. This technique also helps mitigate the significant problem of biomarker heterogeneity in the test tissue.
7	**Treat the disease the patient currently has.**	Although most molecular assays are thought to maintain concordance between primary and metastatic samples, strict data on the concurrence of these assays in a patient's tumor foci over time is not available for all assays. Many oncologists prefer to test the patient's most current tumor (that is, the new metastatic focus), rather than performing molecular testing on a primary tumor sample that may be years old. Be aware of this issue if multiple samples are available for a patient and consult the ordering oncologist if necessary. It is also a good practice to test

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		a stored FFPE tissue with at least one of the originally tested biomarkers, e.g., ER/PgR, to see if the biomolecules have undergone significant degradation with time in storage due to hydrolysis from residual water in the paraffin.
8	**Work with your Referral Lab to assist with specimen selection for sendout testing.**	This may mean reviewing a case to ensure an appropriate test order, communicating with the requesting physician to clarify ambiguous orders, and selecting a paraffin block of tumor and/or normal for the assay.
9	**If you have an in-house molecular laboratory, ask if you can become more involved.**	You can add yourself to the workflow by identifying (“circling”) tumor on the coverslip of the slide for testing, and receiving copies of the test results ordered on your anatomic specimens. In this way, you can learn valuable information about the molecular test ordering patterns of your clinicians, and even suggest ways to streamline ordering or reporting. You may ultimately consider learning to verify in-house molecular assays performed on your samples.

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10	**Stay abreast of new guidelines for testing.**	The CAP has developed the Pathology & Laboratory Quality Center as a forum for establishing consensus guidelines and recommendations through the use of expert panels and collaborations between CAP and other national organizations appropriate to the area of study. Recently the CAP partnered with the Association for Molecular Pathology (AMP) and the International Association for the Study of Lung Cancer (IASLC) to codify recommendations for molecular diagnostics testing in lung cancer associated with targeted therapies. These recommendations were presented at the ASCO Annual Meeting in 2013 and jointly published in *Archives of Pathology & Laboratory Medicine*, the *Journal of Thoracic Oncology*, and the *Journal of Molecular Diagnostics*. Future guidelines in development include molecular testing of colorectal cancer and gastric cancer.

Dr. McCall's suggested articles and resources:

A) **American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer (Unabridged Version)**
Hammond ME, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). *Arch Pathol Lab Med*. 2010 Jul;134(7):e48-72.

Summary: To develop a guideline to improve the accuracy of immunohistochemical (IHC) estrogen receptor (ER) and progesterone receptor (PgR) testing in breast cancer and the utility of these receptors as predictive markers. METHODS: The American Society of

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Clinical Oncology and the College of American Pathologists convened an international Expert Panel that conducted a systematic review and evaluation of the literature in partnership with Cancer Care Ontario and developed recommendations for optimal IHC ER/PgR testing performance. RESULTS: Up to 20% of current IHC determinations of ER and PgR testing worldwide may be inaccurate (false negative or false positive). Most of the issues with testing have occurred because of variation in pre-analytic variables, thresholds for positivity, and interpretation criteria. RECOMMENDATIONS: The Panel recommends that ER and PgR status be determined on all invasive breast cancers and breast cancer recurrences. A testing algorithm that relies on accurate, reproducible assay performance is proposed. Elements to reliably reduce assay variation are specified. It is recommended that ER and PgR assays be considered positive if there are at least 1% positive tumor nuclei in the sample on testing in the presence of expected reactivity of internal (normal epithelial elements) and external controls. The absence of benefit from endocrine therapy for women with ER-negative invasive breast cancers has been confirmed in large overviews of randomized clinical trials.

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PMID: 20586616

B) A Call to Standardize Preanalytic Data Elements for Biospecimens

Robb JA, Gulley ML, Fitzgibbons PL, et al. A Call to Standardize Preanalytic Data Elements for Biospecimens. *Arch Pathol Lab Med*; August 12 2013. [Epub ahead of print]

Summary: Biospecimens must have appropriate clinical annotation (data) to ensure optimal quality for both patient care and research. Clinical preanalytic variables are the focus of this study. Objectives.-To define the essential preanalytic variables (data fields) that should be attached to every collected biospecimen and to provide a complete list of such variables, along with their relative importance, which can vary, depending on downstream use, institutional needs, and information technology capabilities. Design.-The College of American Pathologists Diagnostic Intelligence and Health Information

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Technology Committee sponsored a Biorepository Working Group to develop a ranked list of the preanalytic variables for annotating biospecimens. Members of the working group were experts in anatomic, clinical, and molecular pathology; biobanking; medical informatics; and accreditation. Several members had experience with federal government programs, such as the National Cancer Institute's Biospecimens and Biorepository Branch and National Cancer Institute's Community Cancer Center Program. Potential preanalytic variables were identified and ranked along with available supporting evidence, definitions, and potential negative effects if the variable was not attached to the biospecimen. Additional national and international stakeholders reviewed the draft manuscript. Results.-The ranked listing of 170 preanalytic variables produced can be used as a guide for site-specific implementation into patient care and/or research biorepository processes. Conclusions.-In our collective experience, it is often difficult to choose which of the many preanalytic variables to attach to any specific set of biospecimens used for patient care and/or research. The attached ranked list should aid in the selection of preanalytic variables for a given biospecimen collection.

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PMID: 23937609

C) Biospecimens and Biorepositories for the Community Pathologist

Dash RC, Robb JA, Booker DL, Foo WC, Witte DL, Bry L. Biospecimens and Biorepositories for the Community Pathologist. *Arch Pathol Lab Med*; 136:668-678.

Summary: Pathologists have long served as custodians of human biospecimens collected for diagnostic purposes. Rapid advancements in diagnostic technologies require that pathologists change their practices to optimize patient care. The proper handling of biospecimens creates opportunities for pathologists to improve their diagnoses while assessing prognosis and treatment. In addition, the growing need for high-quality biorepositories represents an opportunity for community pathologists to strengthen their role within the health care team, ensuring that clinical care is not compromised while facilitating research. This article provides a resource to

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community pathologists learning how to create high-quality biorepositories and participating in emerging opportunities in the biorepository field. While a variety of topics are covered to provide breadth of information, the intent is to facilitate a level of understanding that permits community pathologists to make more informed choices in identifying how best their skills and practice may be augmented to address developments in this field.

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PMID: 22646276

D) Preservation of Nucleic Acids and Tissue Morphology in Paraffin Embedded Clinical Samples Comparison of Five Molecular Fixatives

Staff S, Kujala P, Karhu R, et al. Preservation of Nucleic Acids and Tissue Morphology in Paraffin-embedded Clinical Samples: Comparison of Five Molecular Fixatives. *Journal of Clinical Pathology;* 66:807-810.

Summary: Formalin fixation preserves tissue morphology at the expense of macromolecule integrity. Freshly frozen samples are the golden standard for DNA and RNA analyses but require laborious deep-freezing and frozen sectioning for morphological studies. Alternative tissue stabilisation methods are therefore needed. We analysed the preservation of nucleic acids, immunohistochemical staining properties and tissue morphology in paraffin-embedded clinical tissue samples fixed with Z7, RCL2, PAXgene, Allprotect and RNAlater. Formalin-fixed and deep-frozen samples were used as controls. Immunohistochemical analyses showed good preservation of antigenicity in all except Allprotect and RNAlater-fixed samples. RNA quality, based on RNA integrity number value by Bioanalyzer, was comparable with freshly frozen samples only in PAXgene-fixed samples. According to quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analyses, RNA from PAXgene samples yielded results similar to freshly frozen samples. No difference between fixatives was seen in DNA analyses (PCR and real-time PCR). In conclusion, PAXgene seems to be superior to other molecular fixatives and formaldehyde.

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PMID: 23750036

E) Factors Influencing the Degradation of Archival Formalin-Fixed Paraffin Embedded Tissue Sections

Xie R, Chung JY, Ylaya K, et al. Factors Influencing the Degradation of Archival Formalin-Fixed Paraffin-Embedded Tissue Sections. *J Histochem Cytochem*; 59:356-365.

Summary: The loss of antigenicity in archival formalin-fixed paraffin-embedded (FFPE) tissue sections negatively affects both diagnostic histopathology and advanced molecular studies. The mechanisms underlying antigenicity loss in FFPE tissues remain unclear. The authors hypothesize that water is a crucial contributor to protein degradation and decrement of immunoreactivity in FFPE tissues. To test their hypothesis, they examined fixation time, processing time, and humidity of storage environment on protein integrity and antigenicity by immunohistochemistry, Western blotting, and protein extraction. This study revealed that inadequate tissue processing, resulting in retention of endogenous water in tissue sections, results in antigen degradation. Exposure to high humidity during storage results in significant protein degradation and reduced immunoreactivity, and the effects of storage humidity are temperature dependent. Slides stored under vacuum with desiccant do not protect against the effects of residual water from inadequate tissue processing. These results support that the presence of water, both endogenously and exogenously, plays a central role in antigenicity loss. Optimal tissue processing is essential. The parameters of optimal storage of unstained slides remain to be defined, as they are directly affected by preanalytic variables. Nevertheless, minimization of exposure to water is required for antigen preservation in FFPE tissue sections

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Section 3 Molecular Diagnostics for Cancer

3.1 Overview

A) Molecular Testing of Solid Tumors

Igbokwe A, Lopez-Terrada DH. Molecular testing of solid tumors. *Arch Pathol Lab Med.* 2011 January; 135(1):67–82.

Summary: Molecular testing of solid tumors is steadily becoming a vital component of the contemporary anatomic pathologist's armamentarium. These sensitive and specific ancillary tools are useful for confirming ambiguous diagnoses suspected by light microscopy and for guiding therapeutic decisions, assessing prognosis, and monitoring patients for residual neoplastic disease after therapy. Objective—To review current molecular biomarkers and tumor-specific assays most useful in solid tumor testing, specifically of breast, colon, lung, thyroid, and soft tissue tumors, malignant melanoma, and tumors of unknown origin. A few upcoming molecular diagnostic assays that may become standard of care in the near future will also be discussed. Data Sources—Original research articles, review articles, and the authors' personal practice experience. Conclusions—Molecular testing in anatomic pathology is firmly established and will continue to gain ground as the need for more specific diagnoses and new targeted therapies evolve. Knowledge of the more common and clinically relevant molecular tests available for solid tumor diagnosis and management, and their indications and limitations, is necessary if anatomic pathologists are to optimally use these tests and act as consultants for fellow clinicians directly involved in patient care.

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PMID: 21204713

NOTE: Also cited in Section 3.12.3

B) Molecular Staging of Cancer

Technology Assessment Committee. College of American Pathologists. Molecular staging of cancer. December 20, 2010 http://www.cap.org/apps/cap.portal?_nfpb=true&cntvwrPtlt_actionOverride=%2Fportlets%2FcontentViewer%2Fshow&_windowLabel=cntvwrPtlt&cntvwrPtlt{actionForm.contentReference}=committees%2Ftechnology%2Fmsc.html&_state=maximized&_pageLabel=cntvwr. January 19, 2012.

Summary: Molecular methods have been suggested as a way to enhance or replace current morphology-based staging methods. The term 'molecular staging' refers to methods used to ascertain cancer behavior and is often applied to a group of diverse and often unrelated techniques, with little in common except for the fact that they employ non-traditional surgical pathology methods. Developed by the Technology Assessment Committee (TAC), Perspectives on Emerging Technology (POET) reports and white papers are designed to provide pathologists with a high-level summary of a particular emerging technology that is likely to impact their practice in the reasonable future. POET reports help pathologists respond to clinician or patient inquiries about a technology. Its format includes a one-page summary plus select references (e.g., peer-reviewed articles, for further information and research.) Although POETs deliver a short overview of a specific innovative technology, they are not a definitive technology assessment of the techniques used or a "how to" cookbook on implementing a test in a practice. Rather, they are intended to be used as an educational tool leading to a more detailed investigation by the Center, Council on Scientific Affairs, TAC or individual pathologists.

Molecular Staging of Cancer POET Report; POET Reports homepage

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3.2 Choosing Which Molecular Tests to Perform/Interpret

3.2.1 Quick Reference Table: Selected Tests by Tumor Type

Tumor Type	Gene/Loci	Somatic Alteration	Clinical Use	References
Colorectal Adenocarcinoma				
	KRAS codons 12, 13, 61	Mutation	Lack of response to EGFR monoclonal antibodies	1-5, 41
	NRAS codons 12, 13, 61	Mutation	Lack of response to EGFR monoclonal antibodies	2,5
	BRAF	p.V600E mutation	Lack of response to EGFR monoclonal antibodies, MSI stratification, prognostic factor	2,4,6-8, 41
	MLH1	Promoter methylation	Indicates sporadic MSI Tumor	6, 41
Lung Adenocarcinoma				
	EGFR exons 18-21	Mutation	Response to EGFR inhibitors	9-12, 40
	EGFR	p.T790M mutation	Resistance to EGFR inhibitors	13-16, 40
	KRAS codons 12,13,61	Mutation	Exclusion of EGFR mutation	15,17,18, 40

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Lung Adenocarcinoma				
	ALK	Rearrangement	Response to TKI	15,17,19, 40
	ROS1	Rearrangement	Response to TKI	17,20
	MET	Amplification	Resistance to EGFR inhibitors	17,21, 40
Breast Carcinoma				
	HER2/ ERBB2	*Amplification*	*Response to HER2 monoclonal antibodies*	22, 42
Gastric Adenocarcinoma				
	HER2/ ERBB2	Amplification	Response to HER2 monoclonal antibodies	23
Thyroid Carcinoma				
Papillary Thyroid Carcinoma	*BRAF*	p.V600E mutation	Pre-operative FNA diagnosis and prognosis	24
	NRAS, HRAS, KRAS	Mutation	Pre-operative FNA diagnosis	24
	RET-PTC	Rearrangement	Pre-operative FNA diagnosis	24
Follicular Thyroid Carcinoma	*NRAS, HRAS, KRAS*	Mutation	Pre-operative FNA diagnosis	24
	PAX8-PPAR	Rearrangement	Pre-operative FNA diagnosis	24
Melanoma				
Cutaneous & Mucosal	*BRAF* codon 600	Mutation	Response to BRAF inhibitors	25-27
	KIT	Mutation	Response to TKI	28
Uveal	*GNAQ* or *GNA11*	Mutation	Diagnostic	29
	Chromosome 3	Loss (monosomy)	Unfavorable prognosis	30

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GIST				
	KIT	Mutation	Response to TKI	[31]
	PDGFRA	Mutation	Response to TKI	[31]
CNS Neoplasms				
Glioma	*MGMT*	Promoter methylation	Favorable response to alkylating agents	[32]
	IDH1 and *IDH2*	Mutation	Distinguishes reactive gliosis from glioma, favorable prognosis	[33,34]
Oligodendroglioma	Chromosome 1p and 19q	Co-deletion	Favorable prognosis and response to therapy	[35,36]
Pilocytic Astrocytoma	*BRAF*	Duplication/fusion	Diagnostic	[33]
Pleomorphic Xanthoastrocytoma	*BRAF*	p.V600E mutation	Diagnostic	[37]
Cholangiocarcinoma/Pancreatic Carcinoma				
	KRAS codons 12, 13, 61	Mutation	Pre-operative bile duct brushing diagnosis	[38]
Oropharyngeal Squamous Cell Carcinoma				
	HR HPV-related	Positive detection	Favorable response to chemoradiation therapy	[39]

MSI = Microsatellite Instability; TKI = Tyrosine-Kinase Inhibitors; HR HPV= High Risk Human Papillomavirus
This table is meant to be a list of selected tests and is not comprehensive.

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40. Cagle PT, Sholl LM, Lindeman NI, et al. Template for reporting results of biomarker testing of specimens from patients with non-small cell carcinoma of the lung. Arch Pathol Lab Med. 2014 Feb;138(2):171-4.
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42. Fitzgibbons PL, Dillon DA, Alsabeh R, et al. Template for reporting results of biomarker testing of specimens from patients with carcinoma of the breast. Arch Pathol Lab Med. 2014 May;138(5):595-601.

3.2.2 Quick Reference Table by Target Genes

Gene	Tissue of Interest	Clinical Use	AMP Test Menu Link
***ALK* rearrangement**	Lung Adenocarcinoma	Responsive to Crizotinib Diagnostic and positive prognostic factor	Click here
	Anaplastic Large Cell Lymphoma	Diagnostic and positive prognostic factor	Click here
***BRAF* mutation**	Melanoma (p.V600, any variant)	Response to BRAF inhibitors	Click here
	Colorectal Carcinoma (p.V600E)	In microsatellite unstable tumors, indicates sporadic origin (not Lynch Syndrome-related)	Click here
	Colorectal Carcinoma (p.V600E)	Lack of Therapeutic Response to EGFR inhibitors	Click here
	Colorectal Carcinoma (p.V600E)	Poor prognostic factor in microsatellite stable colon cancer	Click here

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***BRAF* mutation**	Thyroid FNA (p.V600E)	Diagnostic for papillary thyroid carcinoma-related neoplasm	Click here
	Papillary Thyroid Carcinoma (p.V600E)	Indicates a more aggressive phenotype, possible targeted therapy	Click here
	Hairy Cell Leukemia (p.V600E)	Used to differentiate from other leukemia/lymphomas	Click here
***CEBPA* mutation**	Acute Myelogenous Leukemia	Good prognostic factor	Click here
***EGFR* Mutation** (exons 18-21)	Lung Adenocarcinoma	Sensitivity to EGFR inhibitors (or rarely resistance-mutation dependent)	Click here
***FLT3* Internal Tandem Duplication**	Acute Myelogenous Leukemia	Poor prognostic factor and aids in therapeutic decision making	Click here
***IDH1* and *IDH2* mutations**	Gliomas	Diagnostic (versus reactive tissue) and positive prognostic marker	Click here
***JAK2* Mutation**	*BCR-ABL* negative myeloproliferative disorders	Diagnostic of Polycythemia Vera, Essential Thrombocythemia or Primary myelofibrosis	Click here
***KIT* Mutation**	Gastrointestinal Intestinal Stromal Tumor	Many mutations responsive to imatinib and related TKIs	Click here
	Melanoma (mucosal & acral mainly, some cutaneous)	Possible response to imatinib	Click here
	Acute Myelogenous Leukemia	AMLs with an inv(16) or t(8;21) and a *KIT* mutation have a higher risk of relapse and worse survival	Click here

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***KIT* Mutation**	Systemic Mastocytosis (p.D816V)	Diagnostic; resistant to imatinib	Click here
***KRAS* Mutation**	Colorectal Carcinoma	Lack of Therapeutic Response to EGFR inhibitors	Click here
	Common Bile Duct Brushings	Usually diagnostic of pancreatic carcinoma or cholangiocarcinoma	Click here
	Lung Adenocarcinoma	Exclusion of EGFR Mutation	Click here
***MPL* Mutation**	*BCR-ABL* negative myeloproliferative disorders	Diagnostic of Essential Thrombocythemia or Primary myelofibrosis	Not available at AMP directory
***NPM1* mutation**	Acute Myelogenous Leukemia	Good prognostic factor and aids in therapeutic decision making	Click here
PIK3CA	Colorectal Adenocarcinoma	Possible Targeted therapy and prognostic factor	Click here
	Breast carcinoma	Possible Targeted therapy and prognostic factor	Not available at AMP directory
ROS1	Lung Adenocarcinoma	Responsive to Crizotinib	Not available at AMP directory

A) Mutations of the BRAF Gene in Human Cancer

Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. *Nature*. 2002 Jun 27;417(6892):949-54.

Summary: Cancers arise owing to the accumulation of mutations in critical genes that alter normal programmes of cell proliferation, differentiation and death. As the first stage of a systematic genome-wide screen for these genes, we have prioritized for analysis signalling pathways in which at least one gene is mutated in human cancer. The RAS RAF MEK ERK MAP kinase pathway mediates cellular responses to growth signals. RAS is mutated to an oncogenic form in about 15% of human cancer. The three RAF genes code for

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cytoplasmic serine/threonine kinases that are regulated by binding RAS. Here we report BRAF somatic missense mutations in 66% of malignant melanomas and at lower frequency in a wide range of human cancers. All mutations are within the kinase domain, with a single substitution (V599E) accounting for 80%. Mutated BRAF proteins have elevated kinase activity and are transforming in NIH3T3 cells. Furthermore, RAS function is not required for the growth of cancer cell lines with the V599E mutation. As BRAF is a serine/threonine kinase that is commonly activated by somatic point mutation in human cancer, it may provide new therapeutic opportunities in malignant melanoma.

Free full text available from *Nature*
PMID: 12068308

B) Laboratory Methods for KRAS Mutation Analysis

Anderson SM. Laboratory methods for KRAS mutation analysis. *Expert Rev Mol Diagn.* 2011 Jul; 11(6): 635-642.

Summary: The determination of KRAS mutational status from tumor samples has become an important tool for patient management in colorectal and non-small-cell lung cancers. Mutations in critical areas of the gene, such as codons 12 and 13, are a negative predictor of response to anti-EGF receptor antibodies in colorectal cancer, and similarly are indicators of resistance to small-molecule tyrosine kinase inhibitors in non-small-cell lung cancer patients. A variety of laboratory methods have been developed to assess mutation status in key regions of the KRAS gene. Many of these methods, including allele-specific PCR, real-time PCR methods with melt-curve analysis, and nucleic acid sequencing techniques, provide the appropriate analytical performance to address tissue heterogeneity in tumor samples. The pathologist plays a key role in this process because assessment of morphological features of the tumor is important prior to molecular analysis. This article provides a summary of the performance characteristics of various molecular testing methods and addresses other key aspects of testing necessary to provide relevant information to help determine appropriate therapy choices.

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Full text available from *Expert Review of Molecular Diagnostics* (USD 60.00)
PMID: 21745016

3.2.3 Setting up a Cancer Testing Molecular Lab

A) Molecular Pathology in Anatomic Pathology Practice: A Review of Basic Principles

Hunt JL. Molecular pathology in anatomic pathology practice: a review of basic principles. *Arch Pathol Lab Med* 2008 Feb; 132:248-260.

Summary: Molecular testing in pathology emerged shortly after polymerase chain reaction became a standard molecular biology assay. Testing efforts began in the clinical laboratories primarily with assays for genetically inherited diseases and assays for clonality in hematologic malignancies. Today, the field has evolved into "molecular diagnostics," which encompasses testing in almost every area of anatomic pathology. Molecular testing is now even making its way definitively into both surgical pathology and cytopathology, although molecular anatomic pathology is still young with few standard tissue-based molecular assays. As more clinically valuable information is gained from molecular pathology testing of tissues, unique challenges are also becoming apparent at the intersection between tissue diagnosis and DNA diagnosis. This review focuses on basic molecular pathology concepts, with particular emphasis on the challenge of tissue-based testing in anatomic pathology.

Free full text available from the CAP's *Archives*
PMID: 18251585

B) Tissue Handling and Specimen Preparation in Surgical Pathology: Issues Concerning the Recovery of Nucleic Acids From Formalin-Fixed, Paraffin-Embedded Tissue

Hewitt SM, Lewis FA, Cao Y, et al. Tissue handling and specimen preparation in surgical pathology: issues concerning the recovery of nucleic acids from formalin-fixed, paraffin-embedded tissue. *Arch Pathol Lab Med.* 2008 Dec; 132(12): 1929-1935.

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Summary: Expression profiling by microarrays and real-time polymerase chain reaction-based assays is a powerful tool for classification and prognostication of disease; however, it remains a research tool, largely reliant on frozen tissue. Limiting the utility of expression profiling is the isolation of quality nucleic acids from formalin-fixed, paraffin-embedded tissue. The collection, handling, and processing of tissue directly impacts the biomolecules that can be recovered from it. High-quality nucleic acids can be obtained from formalin-fixed, paraffin-embedded tissue, but greater attention to all steps in the process of tissue handling and preparation is required. OBJECTIVE: To summarize the current state-of-the-art of preanalytic factors in tissue handling and processing as they impact the quality of RNA obtainable from formalin-fixed, paraffin-embedded tissue. The goals are to provide recommendations that will improve RNA quality for expression profiling from formalin-fixed, paraffin-embedded tissue and highlight areas for additional research. Tissue is an analyte and it must be handled in a standardized fashion to provide consistent results. DATA SOURCES: The literature was reviewed. Consultation with industry and academic leaders in the use of RNA for expression profiling was obtained to identify areas for additional research. CONCLUSIONS: Development of RNA-based assays from formalin-fixed, paraffin-embedded tissue is feasible. Greater attention to tissue handling and processing is essential to improve the quality of biospecimens for the development of robust RNA-based assays. Standardization of procedures and vigorous testing of alternative protocols are required to ensure that these assays function as designed.

Free full text available from the CAP's *Archives*
PMID: 19061293
NOTE: Also cited in Section 1.4

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3.3 Breast Cancer

A) Molecular Pathology of Breast Cancer: The Journey from Traditional Practice Toward Embracing the Complexity of a Molecular Classification

Gruver AM, Portier BP, Tubbs RR. Molecular pathology of breast cancer: the journey from traditional practice toward embracing the complexity of a molecular classification. *Arch Pathol Lab Med*. 2011 May; 135(5):544–557.

Summary: Adenocarcinoma of the breast is the most frequent cancer affecting women in both developed and developing regions of the world. From the moment of clinical presentation until the time of pathologic diagnosis, patients affected by this disease will face daunting questions related to prognosis and treatment options. While improvements in targeted therapies have led to increased patient survival, these same advances have created the imperative to accurately stratify patients to achieve maximum therapeutic efficacy while minimizing side effects. In this evolving era of personalized medicine, there is an ever-increasing need to overcome the limitations of traditional diagnostic practice. Objective.—To summarize the molecular diagnostics traditionally used to guide prognostication and treatment of breast carcinomas, to highlight published data on the molecular classification of these tumors, and to showcase molecular assays that will supplement traditional methods of categorizing the disease. Data Sources.—A review of the literature covering the molecular diagnostics of breast carcinomas with a focus on the gene expression and array studies used to characterize the molecular signatures of the disease. Special emphasis is placed on summarizing evolving technologies useful in the diagnosis and characterization of breast carcinoma. Conclusions.—Available and emerging molecular resources will allow pathologists to provide superior diagnostic, prognostic, and predictive information about individual breast carcinomas. These advances should translate into earlier identification and tailored therapy and should ultimately improve outcome for patients affected by this disease.

Free full text available from the CAP's *Archives*
PMID: 21526953

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B) Current Molecular Diagnostics of Breast Cancer and the Potential Incorporation of MicroRNA

Zoon CK, Starker EQ, Wilson AM, Emmert-Buck MR, Libutti SK, Tangrea MA. Current molecular diagnostics of breast cancer and the potential incorporation of microRNA. *Expert Rev Mol Diagn.* 2009 Jul; 9(5): 455-467.

Summary: Although comprehensive molecular diagnostics and personalized medicine have sparked excitement among researchers and clinicians, they have yet to be fully incorporated into today's standard of care. This is despite the discovery of disease-related oncogenes, tumor-suppressor genes and protein biomarkers, as well as other biological anomalies related to cancer. Each year, new tests are released that could potentially supplement or surpass standard methods of diagnosis, including serum, protein and gene expression analyses. All of these novel approaches have shown great promise, but initial enthusiasm has diminished as difficulties in reproducibility, expense, standardization and proof of significance beyond current protocols have emerged. This review will focus on current and novel molecular diagnostic tools applied to breast cancer with special attention to the exciting new field of microRNA analysis.

Free full text available from PubMed
PMID: 19580430

C) Gene-Expression Signatures in Breast Cancer

Sotiriou C, Pusztai L. Gene-expression signatures in breast cancer. *N Engl J Med*. 2009 Feb 19;360(8):790-800.

Summary: Gene-expression profiling with the use of DNA microarrays allows measurement of thousands of messenger RNA (mRNA) transcripts in a single experiment. Results of such studies have confirmed that breast cancer is not a single disease with variable morphologic features and biomarkers but, rather, a group of molecularly distinct neoplastic disorders. Profiling results also support the hypothesis that estrogen-receptor (ER)–negative and ER-positive breast cancers originate from distinct cell types and point to biologic processes that govern metastatic progression. Moreover, such

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profiling has uncovered molecular signatures that could influence clinical care. In this review, we summarize the results of gene-expression studies that hold the most promise to accelerate the transition between empirical and molecular medicine.

Full text available from *New England Journal of Medicine* (USD 15.00)
PMID: 19228622

D) The Present and Future of Gene Profiling in Breast Cancer
Espinosa E, Gámez-Pozo A, Sánchez-Navarro I, et al. The present and future of gene profiling in breast cancer. *Cancer Metastasis Rev.* 2012 Jun; 31(1-2): 41-46.

Summary: Gene signatures can provide prognostic and predictive information to help in the treatment of early-stage breast cancer. Although many of these signatures have been described, only a few have been properly validated. MammaPrint and OncoType offer prognostic information and identify low-risk patients who do not benefit from adjuvant chemotherapy. With regard to prediction of response, molecular subtypes of breast cancer differ in their sensitivity to chemotherapy, although further studies are needed in this field. Cost, small sample size, and the need to use central laboratories are common limitations to the widespread use of these tools.

Full text available from *Cancer and Metastasis Reviews* (USD 39.95)
PMID: 22124734

E) Gene Expression Profiling: Changing Face of Breast Cancer Classification and Management
Wesolowski R, Ramaswamy B. Gene expression profiling: changing face of breast cancer classification and management. *Gene Expr*. 2011 15(3): 105-115.

Summary: Epithelial breast malignancies are a group of several disease entities that vary in their biology and response to specific therapies. Historically, classification of different molecular types of breast cancer was done through the use of conventional methods such as tumor morphology, grade, and immunophenotyping for

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estrogen, progesterone, and HER-2/neu receptor expression. Such techniques, although helpful, are not sufficient to accurately predict biologic behavior of breast cancers. Over the last several years, much progress has been made in more precise identification of molecular breast cancer subtypes. Such advances hold a great promise in improving estimation of prognosis and assigning most appropriate therapies. Thanks to use of cDNA microarrays expression technology and quantitative reverse transcriptase polymerase chain reaction (RT-PCR), tumors with specific gene expression patterns can now be identified. This process is presently reshaping perceptions of how breast cancer should be classified and treated. Categorization of breast cancers by gene expression is only beginning to make its way into the daily clinical practice and likely will complement, but not replace, the conventional methods of classification.

Full text abstract available from PubMed
PMID: 22268293

3.4 Cervical Cancer

A) Human Papillomavirus DNA Testing (HPV)

Technology Assessment Committee. College of American Pathologists. Human papillomavirus DNA testing (HPV). POET Report developed by CAP's Technology Assessment Committee, updated December 17, 2010. http://www.cap.org/apps/cap.portal?_nfpb=true&cntvwrPtlt_actionOverride=%2Fportlets%2FcontentViewer%2Fshow&_windowLabel=cntvwrPtlt&cntvwrPtlt{actionForm.contentReference}=committees%2Ftechnology%2FHPV.html&_state=maximized&_pageLabel=cntvwr Accessed January 19, 2012.

Summary: High risk HPV (hrHPV) DNA testing is a mainstay component of cervical cancer screening and a cost-effective management tool for equivocal cervical cytology results. The presence of hrHPV DNA is a necessary agent for the development of cervical cancer. Infection with hrHPV is common and, in the vast majority of cases, is self-limited - clearing within 2 years. In a small percentage of cases, the infection becomes persistent and oncogenic

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portions of the hrHPV genome can interact with host cells in ways that lead to genetic and cell regulatory changes. These events may, in turn, lead to carcinogenesis. Therefore, identification of hrHPV DNA is important in the context of screening and triage. Developed by the Technology Assessment Committee (TAC), Perspectives on Emerging Technology (POET) reports and white papers are designed to provide pathologists with a high-level summary of a particular emerging technology that is likely to impact their practice in the reasonable future. POET reports help pathologists respond to clinician or patient inquiries about a technology. Its format includes a one-page summary plus select references (e.g., peer-reviewed articles, for further information and research.) Although POETs deliver a short overview of a specific innovative technology, they are not a definitive technology assessment of the techniques used or a "how to" cookbook on implementing a test in a practice. Rather, they are intended to be used as an educational tool leading to a more detailed investigation by the Center, Council on Scientific Affairs, TAC or individual pathologists.

Human Papillomavirus DNA Testing POET Report; POET Reports homepage

B) American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology Screening Guidelines for the Prevention and Early Detection of Cervical Cancer

Saslow D, Solomon D, Lawson HW, et al. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. *Am J Clin Pathol.* 2012 Apr; 137(4): 516-542.

Summary: An update to the American Cancer Society (ACS) guideline regarding screening for the early detection of cervical precancerous lesions and cancer is presented. The guidelines are based on a systematic evidence review, contributions from 6 working groups, and a recent symposium cosponsored by the ACS, the American Society for Colposcopy and Cervical Pathology, and the American Society for Clinical Pathology, which was attended by 25

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organizations. The new screening recommendations address age-appropriate screening strategies, including the use of cytology and high-risk human papillomavirus (HPV) testing, follow-up (eg, the management of screen positives and screening intervals for screen negatives) of women after screening, the age at which to exit screening, future considerations regarding HPV testing alone as a primary screening approach, and screening strategies for women vaccinated against HPV16 and HPV18 infections.

Free full text available from *American Journal Clinical Pathology*
PMID: 22431528

3.5 Central Nervous System Tumors

3.5.1 Gliomas

A) Molecular Diagnostics of Gliomas

Nikiforova MN, Hamilton RL. Molecular diagnostics of gliomas. *Arch Pathol Lab Med*. 2011 May; 135(5):558–568.

Summary: Gliomas are the most common primary brain tumors of adults and include a variety of histologic types and morphologies. Histologic evaluation remains the gold standard for glioma diagnosis; however, diagnostic difficulty may arise from tumor heterogeneity, overlapping morphologic features, and tumor sampling. Recently, our knowledge about the genetics of these tumors has expanded, and new molecular markers have been developed. Some of these markers have shown diagnostic value, whereas others are useful prognosticators for patient survival and therapeutic response. Objective.—To review the most clinically useful molecular markers and their detection techniques in gliomas. Data Sources.—Review of the pertinent literature and personal experience with the molecular testing in gliomas. Conclusions.—This article provides an overview of the most common molecular markers in neurooncology, including 1p/19q codeletion in oligodendroglial tumors, mutations in the isocitrate dehydrogenase 1 and 2 genes in diffuse gliomas, hypermethylation of the O6-methylguanine-DNA methyltransferase

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gene promoter in glioblastomas and anaplastic gliomas, alterations in the epidermal growth factor receptor and phosphatase and tensin homolog genes in high-grade gliomas, as well as BRAF alterations in pilocytic astrocytomas. Molecular testing of gliomas is increasingly used in routine clinical practice and requires that neuropathologists be familiar with these genetic markers and the molecular diagnostic techniques for their detection.

Full free article available from the CAP's *Archives*
PMID: 21526954

3.6 Gastrointestinal Cancer

3.6.1 Short Presentations in Emerging Concepts: Colorectal Cancer

A) CAP Short Presentations in Emerging Concepts (SPECS): Emerging Concepts in the Workup of Colorectal Cancer [PowerPoint slides]
College of American Pathologists. CAP Short Presentations in Emerging Concepts (SPECS): Emerging Concepts in the Workup of Colorectal Cancer [PowerPoint slides]. Version 1.0.1. Northfield, IL: College of American Pathologists; 2012**.**

Access the slides here

B) Clinical Implementation of KRAS Testing in Metastatic Colorectal Carcinoma: The Pathologist's Perspective
Ross JS. Clinical Implementation of KRAS Testing in Metastatic Colorectal Carcinoma: The Pathologist's Perspective. *Arch Pathol Lab Med*. 2012; 136:1298–307.

Summary: Mutation status of the KRAS gene identifies a distinct disease subtype of metastatic colorectal carcinoma that does not respond to antibody therapeutics targeting the epidermal growth factor receptor. This is currently the only validated marker in metastatic colorectal carcinoma with a clear implication in treatment selection.

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KRAS testing is widely accepted in clinical practice to guide metastatic colorectal carcinoma therapeutic decisions, and there are many commercially available platforms to perform the test. OBJECTIVE: To evaluate the critical role of pathologists in the full implementation of KRAS testing by optimizing tumor tissue collection and fixation procedures and by choosing testing technologies and reliable Clinical Laboratory Improvement Amendments of 1988-certified laboratories to perform the tests. DATA SOURCES: Prospective clinical trials, retrospective studies, and quality assessment and survey reports were identified in the following databases: PubMed, American Society of Clinical Oncology Proceedings (American Society of Clinical Oncology Annual Meeting and Gastrointestinal Cancer Symposium) and European Society for Medical Oncology Proceedings (Annals of Oncology European Society for Medical Oncology Congress and Annals of Oncology World Congress on Gastrointestinal Cancers). CONCLUSIONS: More bona fide standards are needed to address the variety of available test methods, which have different performance characteristics including speed, sensitivity to detect rare mutations, and technical requirements. Refined standards addressing timing of KRAS testing, laboratory performance and accuracy, quality assurance and control, proper tissue collection, and appropriate result reporting would also be greatly beneficial. Pathologists should be aware that the amount of information they need to manage will increase, because future trends and technological advances will enhance the predictive power of diagnostic tests or the scope of the biomarker panels tested routinely across tumor types.

Free full text available from the CAP's *Archives*
PMID: 22272560

C) Value of Mismatch Repair, KRAS, BRAF Mutations in Predicting Recurrence and Benefits from Chemotherapy in Colorectal Cancer

Hutchins G, Southward K, Handley K, et al. Value of Mismatch Repair, KRAS, BRAF Mutations In Predicting Recurrence And Benefits From Chemotherapy In Colorectal Cancer. *J Clin Oncol*. 2011;29:1261-1270.

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Summary: It is uncertain whether modest benefits from adjuvant chemotherapy in stage II colorectal cancer justify the toxicity, cost, and inconvenience. We investigated the usefulness of defective mismatch repair (dMMR), BRAF, and KRAS mutations in predicting tumor recurrence and sensitivity to chemotherapy. PATIENTS AND METHODS: Immunohistochemistry for dMMR and pyrosequencing for KRAS/BRAF were performed for 1,913 patients randomly assigned between fluorouracil and folinic acid chemotherapy and no chemotherapy in the Quick and Simple and Reliable (QUASAR) trial. RESULTS: Twenty-six percent of 695 right-sided colon, 3% of 685 left-sided colon, and 1% of 407 rectal tumors were dMMR. Similarly, 17% of right colon, 2% of left colon, and 2% of rectal tumors were BRAF mutant. KRAS mutant tumors were more evenly distributed: 40% right colon, 28% left colon, and 36% rectal tumors. Recurrence rate for dMMR tumors was half that for MMR-proficient tumors (11% [25 of 218] v 26% [438 of 1,695] recurred; risk ratio [RR], 0.53; 95% CI, 0.40 to 0.70; P < .001). Risk of recurrence was also significantly higher for KRAS mutant than KRAS wild-type tumors (28% [150 of 542] v 21% [219 of 1,041]; RR, 1.40; 95% CI, 1.12 to 1.74; P = .002) but did not differ significantly between BRAF mutant and wild-type tumors (P = .36). No marker predicted benefit from chemotherapy with efficacy not differing significantly by MMR, KRAS, or BRAF status. The prognostic value of MMR and KRAS was similar in the presence and absence of chemotherapy. CONCLUSION: MMR assays identify patients with a low risk of recurrence. KRAS mutational analysis provides useful additional risk stratification to guide use of chemotherapy.

Free full text available from *Journal of Clinical Oncology*
PMID: 21383284

D) NCCN Clinical Practice Guidelines in Oncology: Colon Cancer v 3.2012

National Comprehensive Cancer Network©. NCCN Clinical Practice Guidelines in Oncology: Colon Cancer v 1.2014. http://www.nccn.org/professionals/physician_gls/f_guidelines.asp Accessed September 26, 2013

Free full text available from NCCN website

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E) K-ras Mutations and Benefit from Cetuximab in Advanced Colorectal Cancer

Karapetis CS, Khambata-Ford S, Jonker DJ, et al. K-ras Mutations and Benefit From Cetuximab in Advanced Colorectal Cancer. *N Engl J Med*. 2008;359:1757-1765.

Summary: Treatment with cetuximab, a monoclonal antibody directed against the epidermal growth factor receptor, improves overall and progression-free survival and preserves the quality of life in patients with colorectal cancer that has not responded to chemotherapy. The mutation status of the K-ras gene in the tumor may affect the response to cetuximab and have treatment-independent prognostic value. METHODS: We analyzed tumor samples, obtained from 394 of 572 patients (68.9%) with colorectal cancer who were randomly assigned to receive cetuximab plus best supportive care or best supportive care alone, to look for activating mutations in exon 2 of the K-ras gene. We assessed whether the mutation status of the K-ras gene was associated with survival in the cetuximab and supportive-care groups. RESULTS: Of the tumors evaluated for K-ras mutations, 42.3% had at least one mutation in exon 2 of the gene. The effectiveness of cetuximab was significantly associated with K-ras mutation status (P=0.01 and P<0.001 for the interaction of K-ras mutation status with overall survival and progression-free survival, respectively). In patients with wild-type K-ras tumors, treatment with cetuximab as compared with supportive care alone significantly improved overall survival (median, 9.5 vs. 4.8 months; hazard ratio for death, 0.55; 95% confidence interval [CI], 0.41 to 0.74; P<0.001) and progression-free survival (median, 3.7 months vs. 1.9 months; hazard ratio for progression or death, 0.40; 95% CI, 0.30 to 0.54; P<0.001). Among patients with mutated K-ras tumors, there was no significant difference between those who were treated with cetuximab and those who received supportive care alone with respect to overall survival (hazard ratio, 0.98; P=0.89) or progression-free survival (hazard ratio, 0.99; P=0.96). In the group of patients receiving best supportive care alone, the mutation status of the K-ras gene was not significantly associated with overall survival (hazard ratio for death, 1.01; P=0.97). CONCLUSIONS: Patients with a colorectal tumor bearing mutated K-ras did not benefit from cetuximab, whereas patients with a tumor

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bearing wild-type K-ras did benefit from cetuximab. The mutation status of the K-ras gene had no influence on survival among patients treated with best supportive care alone. (ClinicalTrials.gov number, NCT00079066.)

Free full text available from *New England Journal of Medicine*
PMID: 18946061
NOTE: Also cited in Section 3.6.5

3.6.2 Short Presentations in Emerging Concepts: Lynch Syndrome

A) CAP Short Presentations in Emerging Concepts (SPECS): Emerging Concepts in Colorectal Cancer: Hereditary Nonpolyposis Colorectal Cancer (Lynch Syndrome) [PowerPoint slides]

College of American Pathologists. CAP Short Presentations in Emerging concepts (SPECS): Emerging Concepts in Colorectal Cancer: Hereditary Nonpolyposis Colorectal Cancer (Lynch Syndrome) [PowerPoint slides]. Version 1.0fc1. Northfield, IL: College of American Pathologists; 2012.

Access the slides here

B) Hereditary Nonpolyposis Colorectal Carcinoma and HNPCC-like Families: Problems in Diagnosis, Surveillance, and Management

Lynch HT, Riley BD, Weissman SM, et al. Hereditary nonpolyposis colorectal carcinoma and HNPCC-like families: problems in diagnosis, surveillance, and management. *Cancer* 2004 Jan 1; 100:53-64.
Summary: To the authors' knowledge, hereditary nonpolyposis colorectal carcinoma (HNPCC) is the most commonly occurring hereditary disorder that predisposes to colorectal carcinoma (CRC), accounting for approximately 2-7% of all CRC cases diagnosed in the U.S each year. Its diagnosis is wholly dependent on a meticulously obtained family history of cancer of all anatomic sites, with particular attention to the pattern of cancer distribution within the family. METHODS: The objective of the current study was to illustrate various

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vexing problems that can deter the diagnosis of HNPCC and, ultimately, its management. This was an observational cohort study. Sixteen HNPCC and HNPCC-like families were selected from a large resource of highly extended HNPCC families. High-risk patients were selected from these HNPCC families. An ascertainment bias was imposed by the lack of a population-based data set. Personal interviews and questionnaires were used for data collection. RESULTS: There was an array of difficulties highlighted by limitations in compliance, lack of a clinical or molecular basis for an HNPCC diagnosis, ambiguous DNA findings, problems in genetic counseling, failure to meet Amsterdam or Bethesda criteria, small families, lack of medical and pathologic documentation, poor cooperation of family members and/or their physicians, cultural barriers, economic stress, frequent patient fear and anxiety, perception of insurance discrimination, and limited patient and/or physician knowledge regarding hereditary cancer. CONCLUSIONS: The diagnosis and management of HNPCC is predicated on physician knowledge of its phenotypic and genotypic heterogeneity, in concert with the multifaceted problems that impact on patient compliance.

Free full text available from *Cancer*
PMID: 14692024

C) Recommendations from the EGAPP Working Group: Genetic Testing Strategies in Newly Diagnosed Individuals with Colorectal Cancer Aimed at Reducing Morbidity and Mortality from Lynch Syndrome in Relatives

EGAPP Working Group. Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. *Genet Med* 2009 Jan;11(1):35-41.

Summary: The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group found sufficient evidence to recommend offering genetic testing for Lynch syndrome to individuals with newly diagnosed colorectal cancer to reduce morbidity and mortality in relatives. We found insufficient evidence to recommend a specific genetic testing strategy among the several examined. RATIONALE: Genetic testing to detect Lynch syndrome in individuals

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with newly diagnosed colorectal cancer (CRC) is proposed as a strategy to reduce CRC morbidity and mortality in their relatives (see Clinical Considerations section for definition of Lynch syndrome). The EGAPP Working Group (EWG) constructed a chain of evidence that linked genetic testing for Lynch syndrome in patients with newly diagnosed CRC with improved health outcomes in their relatives. We found that assessing patients who have newly diagnosed CRC with a series of genetic tests could lead to the identification of Lynch syndrome. Relatives of patients with Lynch syndrome could then be offered genetic testing, and, where indicated, colorectal, and possibly endometrial, cancer surveillance, with the expectation of improved health outcome. The EWG concluded that there is moderate certainty that such a testing strategy would provide moderate population benefit. ANALYTIC VALIDITY: The EWG found adequate evidence to conclude that the analytic sensitivity and specificity for preliminary and diagnostic tests were high. CLINICAL VALIDITY: After accounting for the specific technologies and numbers of markers used, the EWG found at least adequate evidence to describe the clinical sensitivity and specificity for three preliminary tests, and for four selected testing strategies. These measures of clinical validity varied with each test and each strategy (see Clinical Considerations section). CLINICAL UTILITY: The EWG found adequate evidence for testing uptake rates, adherence to recommended surveillance activities, number of relatives approachable, harms associated with additional follow-up, and effectiveness of routine colonoscopy. This chain of evidence supported the use of genetic testing strategies to reduce morbidity/mortality in relatives with Lynch syndrome. Several genetic testing strategies were potentially effective, but none was clearly superior. The evidence for or against effectiveness of identifying mismatch repair (MMR) gene mutations in reducing endometrial cancer morbidity or mortality was inadequate. CONTEXTUAL ISSUES: CRC is a common disease responsible for an estimated 52,000 deaths in the United States in 2007. In about 3% of newly diagnosed CRC, the underlying cause is a mutation in a MMR gene (Lynch syndrome) that can be reliably identified with existing laboratory tests. Relatives inheriting the mutation have a high (about 45% by age 70) risk of developing CRC. Evidence suggests these relatives will often accept testing and increased surveillance.

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PMID: 19125126

3.6.3 Gastrointestinal Cancer Review

A) Targeted Therapies and Predictive Markers in Epithelial Malignancies of the Gastrointestinal Tract

McIntire M, Redston M. Targeted Therapies and Predictive Markers in Epithelial Malignancies of the Gastrointestinal Tract. *Arch Pathol Lab Med* 2012 May;136(5):496-503.

Summary: In recent years, there has been a tremendous amount of interest in the development of targeted therapies for the treatment of human cancers. Increased understanding of the specific molecular pathways and driver mutations critical to cancer cell growth have allowed the development of these advanced therapeutics. Among these, inhibitors of the epidermal growth factor receptor and HER2/neu pathways now play a major role in the management of gastrointestinal cancers in addition to other solid malignancies. In colorectal and gastric cancers, the use of epidermal growth factor receptor inhibitors and HER2/neu inhibitors has increased the available treatment options for patients with advanced disease. Objective.—To focus on the current targeted therapies and predictors of response in malignancies of the gastrointestinal tract. Data Sources.—Medical literature searchable on PubMed (US National Library of Medicine) as well as older studies revealed by the literature review were used as the source of data. Conclusion.—Gene testing of critical elements of the pathways targeted by these agents (such as KRAS mutational analysis in colorectal tumors and HER2/neu testing in gastric cancers) allows the ability to predict which patients will respond to these treatments. As the molecular profiling of tumors and our understanding of cancer genomics and epigenetic alterations continues to grow, it is expected that these personalized targeted therapies will form one of the mainstays of gastrointestinal cancer treatment.

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PMID: 22229849

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B) Application of Molecular Techniques in the Diagnosis, Prognosis and Management of Patients with Colorectal Cancer: A Practical Approach

Legolvan MP, Taliano RJ, Resnick MB. Application of molecular techniques in the diagnosis, prognosis and management of patients with colorectal cancer: a practical approach. *Hum Pathol.* 2012 Aug; 43(8): 1157-1168.

Summary: There has been an increasing role for molecular diagnostics in the diagnosis and management of cancer, and colorectal carcinoma is no exception. Recent molecular advances have elucidated 3 broad molecular subtypes of colorectal cancer, including chromosomal instability, microsatellite instability, and cytosine-phosphoguanine island methylator phenotype, which will be discussed. Also, the common syndromes associated with colorectal carcinoma will be reviewed with a focus on the differentiation between Lynch syndrome and microsatellite unstable tumors. Molecular biomarkers for predictive and prognostic markers are also becoming widely used, and due to the clinical use of monoclonal antibodies to the epidermal growth factor receptor, an emphasis is placed on that pathway.

Full text available from *Human Pathology* (USD 31.50)
PMID: 22658275

C) Gastrointestinal Stromal Tumours: Origin and Molecular Oncology

Corless CL, Barnett CM, Heinrich MC. Gastrointestinal stromal tumours: origin and molecular oncology. *Nat Rev Cancer.* 2011 Dec; 11(12): 865-878.

Summary: Gastrointestinal stromal tumours (GISTs) are a paradigm for the development of personalized treatment for cancer patients. The nearly simultaneous discovery of a biomarker that is reflective of their origin and the presence of gain-of-function kinase mutations in these tumours set the stage for more accurate diagnosis and the development of kinase inhibitor therapy. Subsequent studies of genotype and phenotype have led to a molecular classification of

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GIST and to treatment optimization on the basis of molecular subtype. The study of drug-resistant tumours has advanced our understanding of kinase biology, enabling the development of novel kinase inhibitors. Further improvements in GIST treatment may require targeting GIST stem cell populations and/or additional genomic events.

Full text available from *Nature Reviews Cancer* (USD 32.00)
PMID: 22089421

3.6.4 Microsatellite Instability

A) Relevance, Pathogenesis, and Testing Algorithm for Mismatch Repair-Defective Colorectal Carcinomas: A Report of the Association for Molecular Pathology

Funkhouser WK Jr, Lubin IM, Monzon FA, et al. Relevance, pathogenesis, and testing algorithm for mismatch repair-defective colorectal carcinomas: a report of the association for molecular pathology. *J Mol Diagn*. 2012 Mar-Apr;14(2):91-103.

Summary: Loss-of-function defects in DNA mismatch repair (MMR), which manifest as high levels of microsatellite instability (MSI), occur in approximately 15% of all colorectal carcinomas (CRCs). This molecular subset of CRC characterizes patients with better stage-specific prognoses who experience no benefit from 5-fluorouracil chemotherapy. Most MMR-deficient (dMMR) CRCs are sporadic, but 15% to 20% are due to inherited predisposition (Lynch syndrome). High penetrance of CRCs in germline MMR gene mutation carriers emphasizes the importance of accurate diagnosis of Lynch syndrome carriers. Family-based (Amsterdam), patient/family-based (Bethesda), morphology-based, microsatellite-based, and IHC-based screening criteria do not individually detect all germline mutation carriers. These limitations support the use of multiple concurrent tests and the screening of all patients with newly diagnosed CRC. This approach is resource intensive but would increase detection of inherited and de novo germline mutations to guide family screening. Although CRC prognosis and prediction of 5-fluorouracil response are similar in both the Lynch and sporadic dMMR subgroups, these subgroups differ significantly with regard to the implications for family members. We

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recommend that new CRCs should be classified into sporadic MMR-proficient, sporadic dMMR, or Lynch dMMR subgroups. The concurrent use of MSI testing, MMR protein IHC, and BRAF c.1799T>A mutation analysis would detect almost all dMMR CRCs, would classify 94% of all new CRCs into these MMR subgroups, and would guide secondary molecular testing of the remainder.

Free full text available from *Journal of Molecular Diagnostics*
PMID: 22260991

B) Poor Survival Associated with the BRAF V600E Mutation in Microsatellite-stable Colon Cancers

Samowitz WS, Sweeney C, Herrick J, et al. Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. *Cancer Res.* 2005 Jul 15;65(14):6063-9.

Summary: The BRAF V600E mutation has been associated with microsatellite instability and the Cp island methylator phenotype (CIMP) in colon cancer. We evaluated a large population-based sample of individuals with colon cancer to determine its relationship to survival and other clinicopathologic variables. The V600E BRAF mutation was seen in 5% (40 of 803) of microsatellite-stable tumors and 51.8% (43 of 83) of microsatellite-unstable tumors. In microsatellite-stable tumors, this mutation was related to poor survival, CIMP high, advanced American Joint Committee on Cancer (AJCC) stage, and family history of colorectal cancer [odds ratio, 4.23; 95% confidence interval (95% CI), 1.65-10.84]. The poor survival was observed in a univariate analysis of 5-year survival (16.7% versus 60.0%; P <0.01); in an analysis adjusted for age, stage, and tumor site [hazard rate ratio (HRR), 2.97; 95% CI, 2.05-4.32]; in stage-specific, age-adjusted analyses for AJCC stages 2 to 4 (HRR, 4.88, 3.60, and 2.04, respectively); and in Kaplan-Meier survival estimates for AJCC stages 2 to 4 (P < 0.01 for all three stages). Microsatellite-unstable tumors were associated with an excellent 5-year survival whether the V600E mutation was present or absent (76.2% and 75.0%, respectively). We conclude that the BRAF V600E mutation in microsatellite-stable colon cancer is associated with a significantly poorer survival in stages 2 to 4 colon cancer but has no effect on the excellent prognosis of microsatellite-unstable tumors.

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Free full article available from *Cancer Research*
PMID: 16024606

3.6.5 Targeted Rx and Predictive Markers

A) Predictive Molecular Classifiers in Colorectal Cancer

Bohanes P, LaBonte MJ, Winder T, Lenz HJ. Predictive molecular classifiers in colorectal cancer. *Semin Oncol.* 2011 Aug;38(4):576-87.

Summary: The introduction of predictive molecular markers has radically enhanced the identification of which patients may benefit from a given treatment. Despite recent controversies, KRAS mutation is currently the most recognized molecular predictive marker in colorectal cancer (CRC), predicting efficacy of anti-epidermal growth factor receptor (anti-EGFR) antibodies. However, other relevant markers have been reported and claimed to identify patients that will benefit from anti-EGFR therapies. This group of markers includes BRAF mutations, PI3KCA mutations, and loss of PTEN expression. Similarly, molecular markers for cytotoxic agents' efficacy also may predict outcome in patients with CRC. This review aims to summarize the most important predictive molecular classifiers in patients with CRC and further discuss any inconsistent or conflicting findings for these molecular classifiers.

Full article available from *Seminars in Oncology* (USD 31.50)
PMID: 21810517

B) K-Ras Mutations and Benefit From Cetuximab in Advanced Colorectal Cancer

Karapetis CS, Khambata-Ford S, Jonker DJ, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med*. 2008 Oct 23;359(17):1757-65.

Summary: Treatment with cetuximab, a monoclonal antibody directed against the epidermal growth factor receptor, improves overall and progression-free survival and preserves the quality of life in patients with colorectal cancer that has not responded to chemotherapy. The

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mutation status of the K-ras gene in the tumor may affect the response to cetuximab and have treatment-independent prognostic value. METHODS: We analyzed tumor samples, obtained from 394 of 572 patients (68.9%) with colorectal cancer who were randomly assigned to receive cetuximab plus best supportive care or best supportive care alone, to look for activating mutations in exon 2 of the K-ras gene. We assessed whether the mutation status of the K-ras gene was associated with survival in the cetuximab and supportive-care groups. RESULTS: Of the tumors evaluated for K-ras mutations, 42.3% had at least one mutation in exon 2 of the gene. The effectiveness of cetuximab was significantly associated with K-ras mutation status (P=0.01 and P<0.001 for the interaction of K-ras mutation status with overall survival and progression-free survival, respectively). In patients with wild-type K-ras tumors, treatment with cetuximab as compared with supportive care alone significantly improved overall survival (median, 9.5 vs. 4.8 months; hazard ratio for death, 0.55; 95% confidence interval [CI], 0.41 to 0.74; P<0.001) and progression-free survival (median, 3.7 months vs. 1.9 months; hazard ratio for progression or death, 0.40; 95% CI, 0.30 to 0.54; P<0.001). Among patients with mutated K-ras tumors, there was no significant difference between those who were treated with cetuximab and those who received supportive care alone with respect to overall survival (hazard ratio, 0.98; P=0.89) or progression-free survival (hazard ratio, 0.99; P=0.96). In the group of patients receiving best supportive care alone, the mutation status of the K-ras gene was not significantly associated with overall survival (hazard ratio for death, 1.01; P=0.97). CONCLUSIONS: Patients with a colorectal tumor bearing mutated K-ras did not benefit from cetuximab, whereas patients with a tumor bearing wild-type K-ras did benefit from cetuximab. The mutation status of the K-ras gene had no influence on survival among patients treated with best supportive care alone. (ClinicalTrials.gov number, NCT00079066.)

Free full article available from *New England Journal of Medicine*
PMID: 18946061
NOTE: Also cited in Section 3.6.1

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C) Association of KRAS p.G13D Mutation with Outcome in Patients with Chemotherapy-Refractory Metastatic Colorectal Cancer Treated with Cetuximab

De Roock W, Jonker DJ, Di Nicolantonio F, et al. Association of KRAS p.G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. *JAMA.* 2010 Oct 27; 304(16): 1812-1820.

Summary: Patients with metastatic colorectal cancer who have KRAS codon 12- or KRAS codon 13-mutated tumors are presently excluded from treatment with the anti-epidermal growth factor receptor monoclonal antibody cetuximab. OBJECTIVE: To test the hypothesis that KRAS codon 13 mutations are associated with a better outcome after treatment with cetuximab than observed with other KRAS mutations. DESIGN, SETTING, AND PATIENTS: We studied the association between KRAS mutation status (p.G13D vs other KRAS mutations) and response and survival in a pooled data set of 579 patients with chemotherapy-refractory colorectal cancer treated with cetuximab between 2001 and 2008. Patients were included in the CO.17, BOND, MABEL, EMR202600, EVEREST, BABEL, or SALVAGE clinical trials or received off-study treatment. Univariate and multivariate analyses, adjusting for possible prognostic factors and data set, were performed. The effect of the different mutations was studied in vitro by constructing isogenic cell lines with wild-type KRAS, p.G12V, or p.G13D mutant alleles and treating them with cetuximab. MAIN OUTCOME MEASURES: The main efficacy end point was overall survival. Secondary efficacy end points were response rate and progression-free survival. RESULTS: In comparison with patients with other KRAS-mutated tumors, patients with p.G13D-mutated tumors (n = 32) treated with cetuximab had longer overall survival (median, 7.6 [95% confidence interval {CI}, 5.7-20.5] months vs 5.7 [95% CI, 4.9-6.8] months; adjusted hazard ratio [HR], 0.50; 95% CI, 0.31-0.81; P = .005) and longer progression-free survival (median, 4.0 [95% CI, 1.9-6.2] months vs 1.9 [95% CI, 1.8-2.8] months; adjusted HR, 0.51; 95% CI, 0.32-0.81; P = .004). There was a significant interaction between KRAS mutation status (p.G13D vs other KRAS mutations) and overall survival benefit with cetuximab treatment (adjusted HR, 0.30; 95% CI, 0.14-0.67; P = .003). In vitro and mouse model analysis showed that although p.G12V-mutated

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colorectal cells were insensitive to cetuximab, p.G13D-mutated cells were sensitive, as were KRAS wild-type cells. CONCLUSIONS: In this analysis, use of cetuximab was associated with longer overall and progression-free survival among patients with chemotherapy-refractory colorectal cancer with p.G13D-mutated tumors than with other KRAS-mutated tumors. Evaluation of cetuximab therapy in these tumors in prospective randomized trials may be warranted.

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PMID: 20978259

D) KRAS Codon 61, 146 and BRAF Mutations Predict Resistance to Cetuximab Plus Irinotecan in KRAS Codon 12 and 13 Wild-Type Metastatic Colorectal Cancer

Loupakis F, Ruzzo A, Cremolini C, et al. KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. *Br J Cancer*. 2009 Aug 18;101(4):715-21.

Summary: KRAS codons 12 and 13 mutations predict resistance to anti-EGFR monoclonal antibodies (moAbs) in metastatic colorectal cancer. Also, BRAF V600E mutation has been associated with resistance. Additional KRAS mutations are described in CRC. METHODS: We investigated the role of KRAS codons 61 and 146 and BRAF V600E mutations in predicting resistance to cetuximab plus irinotecan in a cohort of KRAS codons 12 and 13 wild-type patients. RESULTS: Among 87 KRAS codons 12 and 13 wild-type patients, KRAS codons 61 and 146 were mutated in 7 and 1 case, respectively. None of mutated patients responded vs 22 of 68 wild type (P=0.096). Eleven patients were not evaluable. KRAS mutations were associated with shorter progression-free survival (PFS, HR: 0.46 P=0.028). None of 13 BRAF-mutated patients responded vs 24 of 74 BRAF wild type (P=0.016). BRAF mutation was associated with a trend towards shorter PFS(HR: 0.59, P=0.073). In the subgroup of BRAF wild-type patients, KRAS codons 61/146 mutations determined a lower response rate (0 vs 37%, P=0.047) and worse PFS (HR: 0.45, P=0.023). Patients bearing KRAS or BRAF mutations had poorer response rate (0 vs 37%, P=0.0005) and PFS (HR: 0.51, P=0.006) compared with KRAS and BRAF wild-type patients. CONCLUSION:

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Assessing KRAS codons 61/146 and BRAF V600E mutations might help optimising the selection of the candidate patients to receive anti-EGFR moAbs.

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E) Wild-Type BRAF is Required For Response to Panitumumab or Cetuximab in Metastatic Colorectal Cancer

Di Nicolantonio F, Martini M, Molinari F, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol*. 2008 Dec 10;26(35):5705-12. Epub 2008 Nov 10.

Summary: Cetuximab or panitumumab are effective in 10% to 20% unselected metastatic colorectal cancer (CRC) patients. KRAS mutations account for approximately 30% to 40% patients who are not responsive. The serine-threonine kinase BRAF is the principal effector of KRAS. We hypothesized that, in KRAS wild-type patients, BRAF mutations could have a predictive/prognostic value. PATIENTS AND METHODS We retrospectively analyzed objective tumor responses, time to progression, overall survival (OS), and the mutational status of KRAS and BRAF in 113 tumors from cetuximab- or panitumumab-treated metastatic CRC patients. The effect of the BRAF V600E mutation on cetuximab or panitumumab response was also assessed using cellular models of CRC. Results KRAS mutations were present in 30% of the patients and were associated with resistance to cetuximab or panitumumab (P = .011). The BRAF V600E mutation was detected in 11 of 79 patients who had wild-type KRAS. None of the BRAF-mutated patients responded to treatment, whereas none of the responders carried BRAF mutations (P = .029). BRAF-mutated patients had significantly shorter progression-free survival (P = .011) and OS (P < .0001) than wild-type patients. In CRC cells, the introduction of BRAF V600E allele impaired the therapeutic effect of cetuximab or panitumumab. Treatment with the BRAF inhibitor sorafenib restored sensitivity to panitumumab or cetuximab of CRC cells carrying the V600E allele. CONCLUSION BRAF wild-type is required for response to panitumumab or cetuximab and could be used to select patients who are eligible for the treatment. Double-hit

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therapies aimed at simultaneous inhibition of epidermal growth factor receptor and BRAF warrant exploration in CRC patients carrying the V600E oncogenic mutation.

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PMID: 19001320

F) Molecular Diagnostics of Colorectal Cancer

Bedeir A, Krasinskas AM. Molecular diagnostics of colorectal cancer. *Arch Pathol Lab Med.* 2011 May; 135(5): 578-587.

Summary: Of all gastrointestinal tract epithelial malignancies, molecular diagnostics has impacted colorectal cancer the most. Molecular testing can detect sporadic and inherited colorectal cancers that arise through the microsatellite instability pathway and can determine the efficacy of targeted drug therapy. OBJECTIVES: To review the microsatellite instability pathway of colorectal carcinoma carcinogenesis and to demonstrate the diagnostic utility of molecular testing in the detection of patients with Lynch syndrome, an inherited disorder of this pathway. Also, to review the significance of detection of KRAS and BRAF gene mutations in predicting the response to anti-epidermal growth factor receptor therapies. DATA SOURCES: This article is based on original publications and review articles that are accessible through the PubMed biomedical database (US National Library of Medicine). CONCLUSIONS: In modern pathology practice, molecular testing is a standard tool that is used to diagnose an inherited colorectal cancer predisposition syndrome (Lynch syndrome) and to help predict outcome and response to therapy for patients with advanced colorectal cancer.

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PMID: 21526956

G) Epidermal Growth Factor Receptor Pathway Mutations and Colorectal Cancer Therapy

Grossmann AH, Samowitz WS. Epidermal growth factor receptor pathway mutations and colorectal cancer therapy. *Arch Pathol Lab Med.* 2011 Oct; 135(10): 1278-1282.

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Summary: Rational anticancer therapy is beginning to expand the practice of surgical pathology beyond a primarily morphologic and immunophenotypic analysis into the molecular arena. Molecular testing of tumors can have both diagnostic and therapeutic value, which guides treatment decisions. This is true for colorectal cancer in which mutations in signaling mediators predict resistance to anti-epidermal growth factor receptor (anti-EGFR) therapy. OBJECTIVE: To review the clinically relevant mutations that currently guide treatment decisions in metastatic colorectal cancer, summarize additional mutations that are expected to improve the prognostic sensitivity of molecular testing, and provide practical suggestions for submitting specimens for molecular analysis. DATA SOURCES: Peer-reviewed literature reporting pertinent clinical trial data, mutation analysis, and molecular mechanisms of drug resistance, as well as comprehensive review articles germane to the topic and published testing recommendations from the College of American Pathologists. CONCLUSIONS: Molecular analysis of colorectal cancer is now mandated before initiation of anti-EGFR therapy and directly impacts treatment options and outcomes. Familiarity with the mutations that determine utility and efficacy of therapy, as well as the importance of careful sample selection, will facilitate appropriate testing and optimize patient care.

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PMID: 21970483

H) BRAF Mutation Testing in Colorectal Cancer

Sharma SG, Gulley ML. BRAF mutation testing in colorectal cancer. *Arch Pathol Lab Med.* 2010 Aug; 134(8): 1225-1228.

Summary: Colorectal cancer is the second most common cause of cancer death in the United States. Understanding the biochemical pathways underlying carcinogenesis has paved the way for more effective treatments and better outcomes. BRAF mutation testing has a role in (1) differentiating sporadic colorectal cancer from Lynch syndrome, (2) identifying cancers lacking BRAF mutation that are more likely to respond to epidermal growth factor receptor inhibitor therapy, and (3) conferring worse prognosis in colorectal cancer that is microsatellite stable. Several analytic methods are available to

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reliably detect BRAF mutations. Real-time polymerase chain reaction identifies the most common BRAF mutation, V600E, in frozen or paraffin-embedded colorectal cancer tissue. Traditional DNA sequencing and the somewhat more-sensitive pyrosequencing method can detect multiple alternative BRAF mutations that are predicted to constitutively activate signaling through the MAPK pathway, promoting tumor growth and survival. Pathologists play an important role in assay validation as well as in consulting with clinicians about indications for testing, ensuring quality of testing, and interpreting results in conjunction with other clinicopathologic factors important in the management of affected patients.

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PMID: 20670148

I) Human Epidermal Growth Factor Receptor 2 Testing in Gastroesophageal Cancer: Correlation Between Immunohistochemistry and Fluorescence in Situ Hybridization

Tafe LJ, Janjigian YY, Zaidinski M, et al. Human epidermal growth factor receptor 2 testing in gastroesophageal cancer: correlation between immunohistochemistry and fluorescence in situ hybridization. *Arch Pathol Lab Med.* 2011 Nov; 135(11): 1460-1465.

Summary: Patients with advanced gastroesophageal cancer have poor survival with current therapy. Human epidermal growth factor receptor 2 (HER2) represents a promising therapeutic target, but the optimal HER2 testing strategy is not yet defined. OBJECTIVES: To evaluate the concordance between immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) and to determine if the American Society of Clinical Oncology/College of American Pathologists HER2 scoring system is applicable to gastroesophageal carcinomas. DESIGN: Formalin-fixed paraffin-embedded tumor samples from patients with advanced stage gastroesophageal cancer were tested by IHC and FISH and scored according to the American Society of Clinical Oncology/College of American Pathologists criteria for breast cancer. Concordance between IHC and FISH was evaluated. A subset of cases was subjected to array comparative genomic hybridization to verify the positive and negative HER2 results. RESULTS: A total of 135 cases with paired IHC and FISH

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results were evaluated. The majority of samples (84%) were biopsies. HER2 amplification was detected in 20 tumors (15%). Using the American Society of Clinical Oncology/College of American Pathologists scoring system, IHC-FISH concordance was 97% for IHC 0, 93% for IHC 1+, and 100% for IHC 3+. Human epidermal growth factor receptor 2 positivity was strongly associated with tumor grade (moderately differentiated > poorly differentiated, P < .001) and histologic subtype (intestinal > diffuse, P = .007). Array comparative genomic hybridization analysis was successful in 31 tumors (14 FISH+ and 17 FISH-). Fluorescence in situ hybridization and array comparative genomic hybridization results were highly concordant in both HER2-positive and HER2-negative groups (93% and 100% concordance, respectively). CONCLUSIONS: Human epidermal growth factor receptor 2 testing in gastroesophageal cancer can be performed using standard breast cancer procedures and the American Society of Clinical Oncology/College of American Pathologists scoring criteria. Although IHC 0 and IHC 3+ provide clear stratification, reliable separation of IHC 1+ and IHC 2+ may be difficult, especially in biopsy samples. The latter 2 groups are best referred to FISH for definitive classification.

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PMID: 22032573

J) Recurrent GNAS Mutations Define an Unexpected Pathway for Pancreatic Cyst Development

Wu J, Matthaei H, Maitra A, et al. Recurrent GNAS mutations define an unexpected pathway for pancreatic cyst development. *Sci Transl Med.* 2011 Jul 20; 3(92): 92ra66.

Summary: More than 2% of the adult U.S. population harbors a pancreatic cyst. These often pose a difficult management problem because conventional criteria cannot always distinguish cysts with malignant potential from those that are innocuous. One of the most common cystic neoplasms of the pancreas, and a bona fide precursor to invasive adenocarcinoma, is called intraductal papillary mucinous neoplasm (IPMN). To help reveal the pathogenesis of these lesions, we purified the DNA from IPMN cyst fluids from 19 patients and searched for mutations in 169 genes commonly altered in human

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cancers. In addition to the expected KRAS mutations, we identified recurrent mutations at codon 201 of GNAS. A larger number (113) of additional IPMNs were then analyzed to determine the prevalence of KRAS and GNAS mutations. In total, we found that GNAS mutations were present in 66% of IPMNs and that either KRAS or GNAS mutations could be identified in 96%. In eight cases, we could investigate invasive adenocarcinomas that developed in association with IPMNs containing GNAS mutations. In seven of these eight cases, the GNAS mutations present in the IPMNs were also found in the invasive lesion. GNAS mutations were not found in other types of cystic neoplasms of the pancreas or in invasive adenocarcinomas not associated with IPMNs. In addition to defining a new pathway for pancreatic neoplasia, these data suggest that GNAS mutations can inform the diagnosis and management of patients with cystic pancreatic lesions.

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PMID: 21775669

3.7 Genitourinary Cancer

A) Emerging Critical Role Of Molecular Testing In Diagnostic Genitourinary Pathology

Netto GJ, Cheng L. Emerging critical role of molecular testing in diagnostic genitourinary pathology. *Arch Pathol Lab Med*. 2012 Apr;136(4):372-90.

Summary: The unprecedented advances in cancer genetics and genomics are rapidly affecting clinical management and diagnostics in solid tumor oncology. Molecular diagnostics is now an integral part of routine clinical management in patients with lung, colon, and breast cancer. In sharp contrast, molecular biomarkers have been largely excluded from current management algorithms of urologic malignancies. Objective.—To discuss promising candidate biomarkers that may soon make their transition to the realm of clinical management of genitourologic malignancies. The need for new treatment alternatives that can improve upon the modest outcome so far in patients with several types of urologic cancer is evident. Well-

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validated prognostic molecular biomarkers that can help clinicians identify patients in need of early aggressive management are lacking. Identifying robust predictive biomarkers that will stratify response to emerging targeted therapeutics is another crucially needed development. A compiled review of salient studies addressing the topic could be helpful in focusing future efforts. Data Sources.—A PubMed (US National Library of Medicine) search for published studies with the following search terms was conducted: molecular, prognostic, targeted therapy, genomics, theranostics and urinary bladder cancer, prostate adenocarcinoma, and renal cell carcinoma. Articles with large cohorts and multivariate analyses were given preference. Conclusions.—Our recent understanding of the complex molecular alterations involved in the development and progression of urologic malignancies is yielding novel diagnostic and prognostic molecular tools and opening the doors for experimental targeted therapies for these prevalent, frequently lethal solid tumors.

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PMID: 22458900

B) Molecular Diagnostics in Urologic Malignancies: A Work in Progress

Netto GJ. Molecular diagnostics in urologic malignancies: a work in progress. *Arch Pathol Lab Med*. 2011 May; 135(5):610–621.

Summary: Molecular diagnostic applications are now an integral part of the management algorithms of several solid tumors, such as breast, colon, and lung. In stark contrast, the current clinical management of urologic malignancies is lagging behind. Clinically robust molecular tests that can identify patients who are more likely to respond to a given targeted agent or even those in need of a more aggressive treatment based on well-validated molecular prognosticators are still lacking. Several promising biomarkers for detection, prognosis, and targeted therapeutics are being evaluated. Objective.—To discuss candidate biomarkers that may soon make the transition to clinical assay for patients in urologic oncology. Data Sources.—Selected original articles published in the PubMed service of the US National Library of Medicine. Conclusions.—Recent understanding of the complex molecular alterations involved in the

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development and progression of urologic malignancies is yielding novel diagnostic and prognostic molecular tools and opening the doors for experimental targeted therapies in these prevalent, frequently lethal solid tumors.

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3.8 Hematopoietic Neoplasms – Quick Reference Table: Genes of Prognostic and Diagnostic Significance

Quick Reference Table: Genes of Prognostic Significance

Disease	Gene	Association	Comment
Myeloid Neoplasia			
AML	FLT3	Intermediate risk in the context of AML with normal karyotype	Refer to the following reviews, referenced below, for more detailed discussion of mutations in these and other genes: Patel 2012, Ofran 2013, Martelli 2013
	NPM1	Favorable risk in the context of AML with normal karyotype and wild-type FTL3	Refer to the following reviews, referenced below, for more detailed discussion of mutations in these and other genes: Patel 2012, Ofran 2013, Martelli 2013

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AML	CEBPA	Double mutations demonstrate favorable risk in the context of AML with normal karyotype	Refer to the following reviews, referenced below, for more detailed discussion of mutations in these and other genes: Patel 2012, Ofran 2013, Martelli 2013
	NF1	Associated with unfavorable cytogenetic risk, including monosomal karyotype. See Boudry-Labis E, et al. Am J Hematology 2013;88:306-311 (not referenced in this review)	Refer to the following reviews, referenced below, for more detailed discussion of mutations in these and other genes: Patel 2012, Ofran 2013, Martelli 2013
	c-KIT	Unfavorable in core-binding factor AML, especially t (8; 21). Some conflicting data has been published, however	Refer to the following reviews, referenced below, for more detailed discussion of mutations in these and other genes: Patel 2012, Ofran 2013, Martelli 2013

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AML	IDH1 & IDH2	Effect on risk varies by study,gene, and specific mutation. Some reports of adverse outcome. Concomitant IDH/NPM1-mutant patients show a very favorable outcome in the context of wild-type FLT3 (see Patel 2012 below)	Refer to the following reviews, referenced below, for more detailed discussion of mutations in these and other genes: Patel 2012, Ofran 2013, Martelli 2013
	TET2	Adverse prognostic risk, including in cases otherwise characterized as intermediate-risk (normal cytogenetics, FLT3-ITD wild-type)	Refer to the following reviews, referenced below, for more detailed discussion of mutations in these and other genes: Patel 2012, Ofran 2013, Martelli 2013
	MLL	Adverse prognostic risk, including in cases otherwise characterized as intermediate-risk (normal cytogenetics, FLT3-ITD wild-type)	Refer to the following reviews, referenced below, for more detailed discussion of mutations in these and other genes: Patel 2012, Ofran 2013, Martelli 2013
	ASXL1	Adverse prognostic risk, including in cases otherwise characterized as intermediate-risk (normal cytogenetics, FLT3-ITD wild-type)	Refer to the following reviews, referenced below, for more detailed discussion of mutations in these and other genes: Patel 2012, Ofran 2013, Martelli 2013

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AML	PHF6	Adverse prognostic risk, including in cases otherwise characterized as intermediate-risk (normal cytogenetics, FLT3-ITD wild-type)	Refer to the following reviews, referenced below, for more detailed discussion of mutations in these and other genes: Patel 2012, Ofran 2013, Martelli 2013
	DNMT3A	Adverse prognostic risk	Refer to the following reviews, referenced below, for more detailed discussion of mutations in these and other genes: Patel 2012, Ofran 2013, Martelli 2013
	JAK2	Adverse prognostic risk in the context of core-binding factor AML	Refer to the following reviews, referenced below, for more detailed discussion of mutations in these and other genes: Patel 2012, Ofran 2013, Martelli 2013
	RUNX1	Adverse prognostic risk in the context of normal karyotype AML	Refer to the following reviews, referenced below, for more detailed discussion of mutations in these and other genes: Patel 2012, Ofran 2013, Martelli 2013

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AML	TP53	Adverse prognostic risk. Associated with complex karyotype and monosomy 17	Refer to the following reviews, referenced below, for more detailed discussion of mutations in these and other genes: Patel 2012, Ofran 2013, Martelli 2013
	SRSF2	Adverse prognostic risk in AML transformed from myeloproliferative neoplasms	Refer to the following reviews, referenced below, for more detailed discussion of mutations in these and other genes: Patel 2012, Ofran 2013, Martelli 2013
	NRAS	Adverse prognostic risk has been reported but may also predict sensitivity to cytarabine therapy	Refer to the following reviews, referenced below, for more detailed discussion of mutations in these and other genes: Patel 2012, Ofran 2013, Martelli 2013
	KRAS	Adverse prognostic risk has been reported but may also predict sensitivity to cytarabine therapy	Refer to the following reviews, referenced below, for more detailed discussion of mutations in these and other genes: Patel 2012, Ofran 2013, Martelli 2013

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AML	WT1	Unclear prognostic impact; conflicting data has been published	Refer to the following reviews, referenced below, for more detailed discussion of mutations in these and other genes: Patel 2012, Ofran 2013, Martelli 2013
MDS	ASXL1	Adverse prognostic risk	Reviewed in Abdel-Wahab 2012 below
	DNMT3A	Unclear prognostic impact; conflicting data has been published	Reviewed in Abdel-Wahab 2012 below
	EZH2	Adverse prognostic risk	Reviewed in Abdel-Wahab 2012 below
	IDH1 & IDH2	Possibly associated with adverse risk	Reviewed in Abdel-Wahab 2012 below
	SF3B1	May confer improved outcome in patients with RARS	Reviewed in Abdel-Wahab 2012 below
	SRSF2	Adverse prognostic risk; associated with RAEB patients	Reviewed in Abdel-Wahab 2012 below
CMML	ASLX1	Adverse prognostic risk	See Itzykson R, et al. J Clin Oncol 2013;31:2428-36 (not referenced in this guide)
	SRSF2	Adverse prognostic risk	See Itzykson R, et al. J Clin Oncol 2013;31:2428-36 (not referenced in this guide)

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JMML	PTPN11	May be associated with adverse prognostic risk	See review in Loh, ML. Hematology 2010;2010:357–362 (not referenced in this guide)
Lymphoid Neoplasia			
B-ALL/LBL	CRLF2	Adverse prognostic risk	See Mullighan, CG. Hematology 2012;2012:389–396 for review (not referenced in this guide)
	IKZF1	Adverse prognostic risk	See Mullighan, CG. Hematology 2012;2012:389–396 for review (not referenced in this guide)
	TP53	Adverse prognostic risk	See Mullighan, CG. Hematology 2012;2012:389–396 for review (not referenced in this guide)
	ERG	Associated with favorable outcome	See Mullighan, CG. Hematology 2012;2012:389–396 for review (not referenced in this guide)
T-ALL/LBL	NOTCH1	Has been reported as a favorable indicator in some pediatric trials. One meta-analysis has shown no effect on survival, however	See Ma 2012 below

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T-ALL/LBL	FBXW7	Has been reported as a predictor of favorable risk	
CLL	IGHV	Somatic hypermutation confers improved prognostic risk. See Szankasi JMD 2010 for proposed diagnostic method	See Rodríguez-Vicente 2013 and Chiorazzi 2012 below for reviews of prognostic markers in CLL
	ATM	Demonstrates adverse prognostic risk in patients with del(11q)	See Rodríguez-Vicente 2013 and Chiorazzi 2012 below for reviews of prognostic markers in CLL
	NOTCH1	Adverse prognostic risk. Associated with unmutated IGHV genes	See Rodríguez-Vicente 2013 and Chiorazzi 2012 below for reviews of prognostic markers in CLL
	MYD88	Other mutated genes reported to confer adverse prognostic risk	See Rodríguez-Vicente 2013 and Chiorazzi 2012 below for reviews of prognostic markers in CLL
	SF3B1	Other mutated genes reported to confer adverse prognostic risk	See Rodríguez-Vicente 2013 and Chiorazzi 2012 below for reviews of prognostic markers in CLL
	TP53	Other mutated genes reported to confer adverse prognostic risk	See Rodríguez-Vicente 2013 and Chiorazzi 2012 below for reviews of prognostic markers

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			in CLL
	ZAP-70	Other mutated genes reported to confer adverse prognostic risk	See Rodríguez-Vicente 2013 and Chiorazzi 2012 below for reviews of prognostic markers in CLL

The genes involved in the pathogenesis and progression of hematopoietic neoplasms is continually growing. A comprehensive list is difficult to assemble and quickly falls out-of-date. The following tables provide a selection of genes for which mutational analysis may be clinically relevant, either for diagnosis or prognostication, even if not routinely available. The decision of whether or not to perform a test for a mutation should include consideration of not only its prognostic or diagnostic significance, but also, the frequency at which the mutation is observed. It may not be effective to perform a test for a mutation that is rarely seen, even if it has large prognostic or diagnostic significance when present.

NOTE: cytogenetic abnormalities are critically important in both diagnostic and prognostic assessment of hematopoietic neoplasms. They are, however, beyond the scope of this molecular resource guide and do not appear below.

Quick Reference Table: Genes of Diagnostic Significance

Disease	Gene	Comment
Myeloid neoplasia		
PV	JAK2	Refer to Kiladjian 2012 below for an excellent overview of the JAK2+ entities, to Scott 2011 for discussion of exon 12 mutations, and to Bench 2013 for a broad description of testing methodologies
ET, PMF	JAK2	V617F gain of function mutation is found in many patients, but not exon 12 mutations, unlike a minority of PV patients

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ET, PMF	MPL	Gain of function mutation W515K/L may be found in JAK2V617F-negative patients
CMML	ASXL1	The presence of mutations in these genes may be useful for the diagnosis of CMML versus non-neoplastic conditions, as recommended in Schnittger 2012 below
	CBL	The presence of mutations in these genes may be useful for the diagnosis of CMML versus non-neoplastic conditions, as recommended in Schnittger 2012 below
	EZH2	The presence of mutations in these genes may be useful for the diagnosis of CMML versus non-neoplastic conditions, as recommended in Schnittger 2012 below
	JAK2	The presence of mutations in these genes may be useful for the diagnosis of CMML versus non-neoplastic conditions, as recommended in Schnittger 2012 below
	KRAS	The presence of mutations in these genes may be useful for the diagnosis of CMML versus non-neoplastic conditions, as recommended in Schnittger 2012 below
	NRAS	The presence of mutations in these genes may be useful for the diagnosis of CMML versus non-neoplastic conditions, as recommended in Schnittger 2012 below
	RUNX1	The presence of mutations in these genes may be useful for the diagnosis of CMML versus non-neoplastic conditions, as recommended in Schnittger 2012 below

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CMML	SRSF219	The presence of mutations in these genes may be useful for the diagnosis of CMML versus non-neoplastic conditions, as recommended in Schnittger 2012 below
	TET2	The presence of mutations in these genes may be useful for the diagnosis of CMML versus non-neoplastic conditions, as recommended in Schnittger 2012 below
JMML	PTPN11	Genetic mutations incorporated into revised diagnostic criteria for JMML, reviewed in Loh, ML. Hematology 2010;2010:357–362 (not referenced in this guide)
	RAS	Genetic mutations incorporated into revised diagnostic criteria for JMML, reviewed in Loh, ML. Hematology 2010;2010:357–362 (not referenced in this guide)
	NF1 (germline mutations)	Genetic mutations incorporated into revised diagnostic criteria for JMML, reviewed in Loh, ML. Hematology 2010;2010:357–362 (not referenced in this guide)
Lymphoid neoplasia		
B-cell malignancy	IGH	Useful for determination of suspected clonality, clonal interrelatedness of separate neoplasms, following minimal residual disease, etc. Please refer to Evans 2007, Bagg 2008, and Langerak 2012 below
T-cell malignancy	TCRB, TCRG	Useful for determination of suspected clonality, clonal interrelatedness of separate neoplasms, following minimal residual disease, etc. Please refer to Brüggemann 2007, Langerak 2012, Dereure 2006, and Cushman-Vokoun 2010 below

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HCL	BRAF	V600E mutation in the great majority of cases of hairy cell leukemia but lacking in other lymphoid neoplasms
WM/LPL	MYD88	L265P mutation supports a diagnosis of Waldenstroms macroglobulinemia/lymphoplasmacytic lymphoma over plasma cell neoplasms, marginal zone lymphoma and chronic lymphocytic leukemia/small cell lymphoma

3.8.1 Myeloid Neoplasia

3.8.1.1 Chronic Myelogenous Leukemia

A) BCR-ABL1 Kinase Domain Mutations: Methodology and Clinical Evaluation

Alikian M, Gerrard G, Subramanian PG, et al. BCR-ABL1 kinase domain mutations: methodology and clinical evaluation. *Am J Hematol.* 2012 Mar; 87(3): 298-304.

Summary: The introduction of tyrosine kinase inhibitors (TKIs), starting with imatinib and followed by second and third generation TKIs, has significantly changed the clinical management of patients with chronic myeloid leukemia (CML). Despite their unprecedented clinical success, a proportion of patients fail to achieve complete cytogenetic remission by 12 months of treatment (primary resistance) while others experience progressive resistance after an initial response (secondary resistance). BCR-ABL1 kinase domain (KD) mutations have been detected in a proportion of patients at the time of treatment failure, and therefore their identification and monitoring plays an important role in therapeutic decisions particularly when switching TKIs. When monitoring KD mutations in a clinical laboratory, the choice of method should take into account turnaround time, cost, sensitivity, specificity, and ability to accurately quantify the size of the mutant clone. In this article, we describe in a "manual" style the

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methods most widely used in our laboratory to monitor KD mutations in patients with CML including direct sequencing, D-HPLC, and pyrosequencing. Advantages, disadvantages, interpretation of results, and their clinical applications are reviewed for each method.

Free full text available *American Journal Hematology*
PMID: 22231203

B) BCR-ABL1 Compound Mutations in Tyrosine Kinase Inhibitor-Resistant CML: Frequency and Clonal Relationships

Khorashad JS, Kelley TW, Szankasi P, et al. BCR-ABL1 compound mutations in tyrosine kinase inhibitor-resistant CML: frequency and clonal relationships. *Blood.* 2013 Jan 17; 121(3): 489-498.

Summary: BCR-ABL1 compound mutations can confer high-level resistance to imatinib and other ABL1 tyrosine kinase inhibitors (TKIs). The third-generation ABL1 TKI ponatinib is effective against BCR-ABL1 point mutants individually, but remains vulnerable to certain BCR-ABL1 compound mutants. To determine the frequency of compound mutations among chronic myeloid leukemia patients on ABL1 TKI therapy, in the present study, we examined a collection of patient samples (N = 47) with clear evidence of 2 BCR-ABL1 kinase domain mutations by direct sequencing. Using a cloning and sequencing method, we found that 70% (33/47) of double mutations detected by direct sequencing were compound mutations. Sequential, branching, and parallel routes to compound mutations were common. In addition, our approach revealed individual and compound mutations not detectable by direct sequencing. The frequency of clones harboring compound mutations with more than 2 missense mutations was low (10%), whereas the likelihood of silent mutations increased disproportionately with the total number of mutations per clone, suggesting a limited tolerance for BCR-ABL1 kinase domain missense mutations. We conclude that compound mutations are common in patients with sequencing evidence for 2 BCR-ABL1 mutations and frequently reflect a highly complex clonal network, the evolution of which may be limited by the negative impact of missense mutations on kinase function.

Full text available from *Blood* (USD 35.00)

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PMID: 23223358

C) Standardized Definitions of Molecular Response in Chronic Myeloid Leukemia

Cross NC, White HE, Müller MC, Saglio G, Hochhaus A. Standardized definitions of molecular response in chronic myeloid leukemia. *Leukemia.* 2012 Oct; 26(10): 2172-2175.

Summary: The International Randomized Study of Interferon and STI571 (IRIS) demonstrated long-term cytogenetic responses in patients with chronic-phase chronic myeloid leukemia (CML-CP) treated with the tyrosine kinase inhibitor (TKI) imatinib. However, deep molecular responses (MRs), as measured by reductions in BCR-ABL transcript levels below the threshold of major MR, were achieved only by a small proportion of patients. With the advent of the second-generation TKIs nilotinib and dasatinib for the treatment of patients with newly diagnosed CML-CP, the proportion of patients who achieve the deepest levels of MR is likely to increase significantly. With these changes, the potential for patient eligibility in TKI cessations studies is becoming a more widely discussed topic and area for research. These developments highlight the need for robust, standardized and workable definitions of deep MRs. Specifically, it is critical that the measurement of MR is standardized in a manner to withstand both intra- and inter-laboratory variability, as well as new methodological developments. This review summarizes the relevant clinical background and proposes a framework within which standardization of MR can be taken forward.

Full text available from *Leukemia* (USD 32.00)
PMID: 22504141

D) Molecular Resistance: An Early Indicator for Treatment Change?

Fava C, Kantarjian H, Cortes J. Molecular resistance: an early indicator for treatment change? *Clin Lymphoma Myeloma Leuk.* 2012 Apr; 12(2): 79-87.

Summary: Vigilant monitoring of a patient's response to current treatment is imperative to the management of chronic myeloid leukemia (CML). Early identification of treatment failure may increase

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the probability that alternative therapy will be effective. This review discusses the use of molecular monitoring in the timely detection of failure of imatinib treatment. Changes in the levels of BCR-ABL transcripts are predictive of response or relapse. Patients achieving a major molecular response (MMR) within 12 months of treatment may experience longer cytogenetic remission. Accumulating evidence also suggests that lower transcript levels observed </= 6 months after the start of treatment are associated with improved patient outcomes. For patients with primary or secondary imatinib resistance (or intolerance), dasatinib or nilotinib may be prescribed. These agents have demonstrated activity in patients harboring imatinib-resistant BCR-ABL mutations, except for the T315I substitution.

Full text available from *Clinical Lymphoma, Myeloma and Leukemia* (USD 31.50)
PMID: 22285607

E) Translating Trial-Based Molecular Monitoring into Clinical Practice: Importance of International Standards and Practical Considerations for Community Practitioners

Akard LP, Wang YL. Translating trial-based molecular monitoring into clinical practice: importance of international standards and practical considerations for community practitioners. *Clin Lymphoma Myeloma Leuk.* 2011 Oct; 11(5): 385-395.

Summary: The success of tyrosine kinase inhibition of the BCR-ABL fusion gene with imatinib in the treatment of chronic myeloid leukemia (CML) has resulted in the use of molecular detection techniques for routine clinical management. Current clinical guidelines recommend the use of molecular testing of BCR-ABL transcript levels by quantitative real-time transcriptase polymerase chain reaction (qRT-PCR) every 3 to 6 months. However, qRT-PCR methods have not yet been standardized, particularly in the United States, where most patients are initially treated outside of academic practices. The lack of standard methods for molecular monitoring has resulted in the failure to follow National Comprehensive Cancer Network and European LeukemiaNet guideline recommendations and in the misinterpretation of test results. Standardization of molecular monitoring methods and adherence to guideline recommendations are important for optimal

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patient management. In this article, we provide an update on the current clinical trial results by using the molecular technique to monitor patient response. Current problems and efforts in standardizing the qRT-PCR technique and reporting are reviewed. We provide examples of potential problems of various reference laboratory reports and present recommendations for assessing molecular test results. These recommendations seem particularly important because nilotinib and dasatinib appear to have improved the molecular response in the initial treatment of CML.

Full text available from *Clinical Lymphoma Myeloma and Leukemia*
(USD 31.50)
PMID: 21723805

3.8.1.2 BCR-ABL1-negative Myeloproliferative Neoplasms

3.8.1.2.1 Short Presentations in Emerging Concepts: JAK2

A) CAP Short Presentations in Emerging Concepts (SPECS): Emerging Concepts in the Workup of Polycythemia and Thrombocythemia: *JAK2* [PowerPoint slides]
College of American Pathologists. CAP Short Presentations in Emerging concepts (SPECS): Emerging Concepts in the Workup of Polycythemia and Thrombocythemia: JAK2 [PowerPoint slides]. Version 0.3. Northfield, IL: College of American Pathologists; 2012.

Access slides here

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3.8.1.2.2 Articles on BCR-ABL1-Negative Myeloproliferative Neoplasms

A) Molecular Diagnosis of the Myeloproliferative Neoplasms: UK Guidelines for the Detection of JAK2 V617F and Other Relevant Mutations

Bench AJ, White HE, Foroni L, et al. Molecular diagnosis of the myeloproliferative neoplasms: UK guidelines for the detection of JAK2 V617F and other relevant mutations. *Br J Haematol.* 2013 Jan; 160(1): 25-34.

Summary: Molecular genetic assays for the detection of the JAK2 V617F (c.1849G>T) and other pathogenetic mutations within JAK2 exon 12 and MPL exon 10 are part of the routine diagnostic workup for patients presenting with erythrocytosis, thrombocytosis or otherwise suspected to have a myeloproliferative neoplasm. A wide choice of techniques are available for the detection of these mutations, leading to potential difficulties for clinical laboratories in deciding upon the most appropriate assay, which can lead to problems with inter-laboratory standardization. Here, we discuss the most important issues for a clinical diagnostic laboratory in choosing a technique, particularly for detection of the JAK2 V617F mutation at diagnosis. The JAK2 V617F detection assay should be both specific and sensitive enough to detect a mutant allele burden as low as 1-3%. Indeed, the use of sensitive assays increases the detection rate of the JAK2 V617F mutation within myeloproliferative neoplasms. Given their diagnostic relevance, it is also beneficial and relatively straightforward to screen JAK2 V617F negative patients for JAK2 exon 12 mutations (in the case of erythrocytosis) or MPL exon 10 mutations (thrombocytosis or myelofibrosis) using appropriate assays. Molecular results should be considered in the context of clinical findings and other haematological or laboratory results.

Free full text available from *British Journal of Haematology*
PMID: 23057517

B) The JAK2 Exon 12 Mutations: A Comprehensive Review

Scott LM. The JAK2 exon 12 mutations: a comprehensive review. *Am J Hematol.* 2011 Aug; 86(8): 668-676.

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Summary: A variety of acquired mutations targeting JAK2 exon 12 are present in those patients with the myeloproliferative neoplasm, polycythemia vera, that lack the more common JAK2V617F mutation. Both mutation types perturb erythropoiesis, with individuals presenting with a raised hematocrit, reduced serum erythropoietin levels, and erythropoietin-independent erythroid progenitor cells. However, there are also phenotypic differences that, until recently, precluded a significant proportion of patients with a JAK2 exon 12 mutation from receiving an appropriate diagnosis. Here, we review the literature published on the JAK2 exon 12 mutations and compare the biology associated with these mutations with that of JAK2V617F.

Free full text available from *American Journal of Hematology*
PMID: 21674578

C) Molecular Analyses of 15,542 Patients with Suspected BCR-ABL1-Negative Myeloproliferative Disorders Allow to Develop a Stepwise Diagnostic Workflow

Schnittger S, Bacher U, Eder C, et al. Molecular analyses of 15,542 patients with suspected BCR-ABL1-negative myeloproliferative disorders allow to develop a stepwise diagnostic workflow. *Haematologica.* 2012 Oct; 97(10): 1582-1585.

Summary: We investigated 15,542 patients with suspected BCR-ABL1- negative myeloproliferative or myelodysplastic/myeloproliferative neoplasm (including 359 chronic myelomonocytic leukemia) by a molecular marker set. JAK2V617F was detected in the suspected categories as follows: polycythemia vera 88.3%, primary myelofibrosis 53.8%, essential thrombocythemia 50.2%, and not further classifiable myeloproliferative neoplasms 38.0%. JAK2 exon 12 mutations were detected in 40.0% JAK2V617F-negative suspected polycythemia vera, MPLW515 mutations in 13.2%JAK2V617F-negative primary myelofibrosis and 7.1% JAK2V617F-negative essential thrombocythemia. TET2 mutations were distributed across all entities but were most frequent in suspected chronic myelomonocytic leukemia (77.8%). CBL mutations were identified in suspected chronic myelomonocytic leukemia (13.9%), primary myelofibrosis (8.0%), and not further classifiable

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myeloproliferative neoplasm (7.0%). This leads to a stepwise workflow for suspected myeloproliferative neoplasms starting with JAK2V617F and investigating JAK2V617F-negative patients for JAK2 exon 12 or MPL mutations, respectively. In cases in which a myeloproliferative neoplasm cannot be established, analysis for TET2, CBL and EZH2 mutations may be indicated.

Free full text available in PubMed
PMID: 22511494

D) The Spectrum of JAK2-Positive Myeloproliferative Neoplasms

Kiladjian JJ. The spectrum of JAK2-positive myeloproliferative neoplasms. *Hematology Am Soc Hematol Educ Program.* 2012 2012:561-566.

Summary: The discovery of the JAK2V617F mutation triggered an unexpected flowering of basic and clinical studies in the field of myeloproliferative neoplasms (MPNs), resulting after just a few years in an exceptional amount of new information. One important consequence of those new findings was the modification of the World Health Organization classification and diagnostic algorithms for these diseases, which is still based on the original concept developed by William Dameshek in 1951 and keeps distinct entities under the umbrella of classical Philadelphia-negative MPNs. These MPNs are essential thrombocythemia, polycythemia vera, and primary myelofibrosis. Could a new molecular classification be a better tool to manage MPN patients? Several studies have shown that essential thrombocythemia and primary myelofibrosis can be divided into distinct subtypes based on the presence of the JAK2V617F mutation. Can we now define JAK2-positive diseases to depict a distinct entity from JAK2-negative MPNs? This chapter reviews the significance of JAK2 mutation positivity in the diagnosis, prognosis, and therapy of MPNs.

Free full text available in *Hematology*
PMID: 23233635

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E) Genetic and Epigenetic Complexity in Myeloproliferative Neoplasms

Cross NC. Genetic and epigenetic complexity in myeloproliferative neoplasms. *Hematology Am Soc Hematol Educ Program*. 2011;2011:208-14.

Summary: The past 7 years have witnessed remarkable progress in our understanding of the genetics of BCR-ABL-negative myeloproliferative neoplasms (MPNs) and has revealed layers of unexpected complexity. Deregulation of JAK2 signaling has emerged as a central feature, but despite having biological activities that recapitulate the cardinal features MPNs in model systems, JAK2 mutations are often secondary events. Several other mutated genes have been identified with a common theme of involvement in the epigenetic control of gene expression. Remarkably, the somatic mutations identified to date do not seem to be acquired in any preferred order, and it is possible that the disease-initiating events remain to be identified. The finding of complex clonal hierarchies in many cases suggests genetic instability that, in principle, may be inherited or acquired. A common haplotype has been identified that is strongly associated with the acquisition of JAK2 mutations, but the cause of relatively high-penetrance familial predisposition to MPNs remains elusive. This review summarizes the established facts relating to the genetics of MPNs, but highlights recent findings and areas of controversy.

Free full text available from *Hematology*

PMID: 22160036

F) Pathology Consultation on Myeloproliferative Neoplasms

Schmidt AE. Pathology Consultation on Myeloproliferative Neoplasms. Oh ST; for the Education Committee of the Academy of Clinical Laboratory Physicians and Scientists. *Am J Clin Pathol*. 2012 Jul;138(1):12-19.

Summary: In 2008, the World Health Organization (WHO) revised the classification system for myeloproliferative neoplasms (MPNs). MPNs include chronic myelogenous leukemia, essential thrombocythemia, polycythemia vera, primary myelofibrosis, and several other disorders. The newer classification system incorporates mutations discovered in

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the JAK2 and MPL genes. The importance of understanding the role of mutations in JAK2, MPL, and other genes that have been discovered in MPNs is highlighted by the change in the 2008 WHO MPN classification system. Moreover, the development of highly specific inhibitors of JAK2 further stresses the importance of molecular testing in MPN diagnosis and prognosis.

Free full text from *American Journal of Clinical Pathology*
PMID: 22706852

G) JAK Inhibitors for Myeloproliferative Neoplasms: Clarifying Facts from Myths

Tefferi A. JAK inhibitors for myeloproliferative neoplasms: clarifying facts from myths. *Blood*. 2012 Mar 22;119(12):2721-30. Epub 2012 Jan 25.

Summary: On November 16, 2011, the Food and Drug Administration approved ruxolitinib (a JAK1 and JAK2 inhibitor) for use in the treatment of high and intermediate risk myelofibrosis. This is welcome news for those patients in whom such therapy is indicated and treatment benefit outweighs attendant risk. The question is who are these patients, what should they expect in terms of both short-term effects and long-term impact, and why would they choose ruxolitinib over other JAK inhibitors that are freely available for use in a research setting. Ruxolitinib and most other JAK inhibitors exert a salutary effect on constitutional symptoms and splenomegaly but have yet to produce histopathologic or cytogenetic remissions, reverse bone marrow fibrosis, or improve survival over best supportive care. Furthermore, the palliative value of JAK inhibitors is diminished by notable side effects, including anemia, thrombocytopenia, gastrointestinal disturbances, metabolic abnormalities, peripheral neuropathy, and hyperacute relapse of symptoms during treatment discontinuation. Therefore, risk-benefit balance favors use of currently available JAK inhibitors in only a select group of patients with myelofibrosis, and their potential value in polycythemia vera, outside of special circumstances (eg, intractable pruritus), is undermined by the absence of evidence for a disease-modifying effect and presence of arguably superior alternatives.

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Free full text available from *Blood*
PMID: 22279053

H) Decrease in JAK2 V617F Allele Burden is Not a Prerequisite to Clinical Response in Patients with Polycythemia Vera

Kuriakose E, Vandris K, Wang YL, et al. Decrease in JAK2 V617F allele burden is not a prerequisite to clinical response in patients with polycythemia vera. *Haematologica*. 2012 Apr;97(4):538-42.

Summary: Although reduction in the JAK2(V617F) allele burden (%V617F) has been suggested as a criterion for defining disease response to cytoreductive therapy in polycythemia vera, its value as a response monitor is unclear. The purpose of this study is to determine whether a reduction in %V617F in polycythemia vera is a prerequisite to achieving hematologic remission in response to cytoreductive therapy. DESIGN AND METHODS: We compared the clinical and hematologic responses to change in %V617F (molecular response) in 73 patients with polycythemia vera treated with either interferon (rIFNα-2b: 28, Peg-rIFNα-2a: 18) or non-interferon drugs (n=27), which included hydroxyurea (n=8), imatinib (n=12), dasatinib (n=5), busulfan (n=1), and radioactive phosphorus (n=1). Hematologic response evaluation employed Polycythemia Vera Study Group criteria, and molecular response evaluation, European Leukemia Net criteria. RESULTS: Of the 46 treated with interferon, 41 (89.1%) had a hematologic response, whereas only 7 (15.2%) had a partial molecular response. Of the 27 who received non-interferon treatments, 16 (59.3%) had a hematologic response, but only 2 (7.4%) had a molecular response. Median duration of follow up was 2.8 years. Statistical agreement between hematologic response and molecular response was poor in all treatment groups. CONCLUSIONS: Generally, hematologic response was not accompanied by molecular response. Therefore, a quantitative change in %V617F is not required for clinical response in patients with polycythemia vera.

Free full text available from PubMed
PMID: 22102708

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I) Relevance of JAK2V617F Positivity to Hematological Diseases--Survey of Samples from a Clinical Genetics Laboratory

Zhao W, Gao R, Lee J, et al. Relevance of JAK2V617F positivity to hematological diseases--survey of samples from a clinical genetics laboratory. *J Hematol Oncol*. 2011 Jan 14;4:4.

Summary: JAK2V617F is found in the majority of patients with Ph-myeloproliferative neoplasms (MPNs) and has become a valuable marker for diagnosis of MPNs. However, it has also been found in many other hematological diseases, and some studies even detected the presence of JAK2V617F in normal blood samples. This casts doubt on the primary role of JAK2V617F in the pathogenesis of MPNs and its diagnostic value. METHODS: In the present study, we analyzed JAK2V617F positivity with 232 normal blood samples and 2663 patient blood, bone marrow, and amniotic fluid specimens obtained from a clinical genetics laboratory by using a simple DNA extraction method and a sensitive nested allele-specific PCR strategy. RESULTS: We found JAK2V617F present in the majority (78%) of MPN patients and in a small fraction (1.8-8.7%) of patients with other specific hematological diseases but not at all in normal healthy donors or patients with non-hematological diseases. We also revealed associations of JAK2V617F with novel as well as known chromosomal abnormalities. CONCLUSIONS: Our study suggests that JAK2V617F positivity is associated with specific hematological malignancies and is an excellent diagnostic marker for MPNs. The data also indicate that the nested allele-specific PCR method provides clinically relevant information and should be conducted for all cases suspected of having MPNs as well as for other related diseases.

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PMID: 21235771

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3.8.1.3 Myelodysplastic Syndromes and MDS/MPN Overlap Neoplasms

A) Interpreting New Molecular Genetics in Myelodysplastic Syndromes

Abdel-Wahab O, Figueroa ME. Interpreting new molecular genetics in myelodysplastic syndromes. *Hematology Am Soc Hematol Educ Program.* 2012 2012:56-64.

Summary: The myelodysplastic syndromes (MDS) are a clinically and cytogenetically heterogeneous group of clonal diseases characterized by ineffective hematopoiesis, peripheral blood cytopenias, and an increased risk of progression to acute myeloid leukemia. The precise molecular mechanisms behind the development of MDS have remained elusive; however, the distinct sensitivity of this disease to DNA methyltransferase inhibitors and the presence of markedly abnormal epigenetic profiles suggested the existence of an epigenetic mechanism underlying the disease. Recently, the advent of new technologies for the detection of genetic abnormalities has led to the description of a set of novel recurrent mutations in patients with this disease. The majority of these novel mutations have been described in genes encoding different components of the epigenetic machinery, many of which are associated with distinct clinical outcomes. Finally, mutations in mRNA splicing genes have also been described recently in MDS, underscoring the molecular complexity that underlies the development of this heterogeneous disease.

Free full text available from *Hematology*
PMID: 23233561

3.8.1.4 Acute Myeloid Leukemia

A) With AML Genetic Profiling, It Takes All Kinds

Titus KL. With AML genetic profiling, it takes all kinds. *CAP TODAY.* 2012 June.

Summary: Genetic mutations—primarily FLT3, CEBPA, and NPM1—have been part of the acute myeloid leukemia picture since

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2008, says Dr. Gail Vance, whose lab at Indiana University tests for the mutations in cases of AML with normal chromosomes. A recent ECOG trial found those three to have some company. The phase three clinical trial, E1900, provides the most nuanced view of AML genetic profiles to date, with researchers performing a mutational analysis of 18 genes in 398 AML patients under age 60.

Free full article available from *CAP TODAY*

B) Molecular Diagnosis of Acute Myeloid Leukemia: Present and Future Challenges

Czuchlewski D, Vasef M. PHC Webinar. Molecular diagnosis of acute myeloid leukemia: present and future challenges. [Webinar]. May 31, 2012. https://www1.gotomeeting.com/register/396549136. Accessed July 10, 2012.

Summary: While personalized molecular approaches to solid tumors continue to evolve, genetic information has long been critical in the diagnosis and classification of acute myeloid leukemia (AML). This webinar will review the development of current diagnostic tools that further refine the distinct subtypes of AML, with a practical focus on questions likely to occur in the setting of a general pathology practice, including: How can molecular data be used to guide patient care? Which patients need molecular testing? Which genes should be evaluated and by what methods? In addition, the presenters will give an update to the 2008 WHO classification, including new information on established targets and novel gene mutations likely to impact clinical practice in the near future. Finally, we discuss the revolutionary changes coming to molecular diagnosis of AML in the dawning era of next-generation sequencing.

Archived webinar available for free

C) How Do Novel Molecular Genetic Markers Influence Treatment Decisions in Acute Myeloid Leukemia?

Patel JP, Levine RL. How do novel molecular genetic markers influence treatment decisions in acute myeloid leukemia? *Hematology Am Soc Hematol Educ Program.* 2012 2012:28-34.

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Summary: Acute myeloid leukemia (AML) is the most common acute leukemia diagnosed in adults, and the majority of patients with AML die from relapsed disease. Although many studies over the past 4 decades have identified disease alleles in AML, recent genome-wide and candidate gene studies have identified additional recurrent somatic mutations in AML patients with biologic, clinical, and therapeutic importance. Herein we review our current understanding of the molecular pathogenesis of AML and discuss how mutational profiling can be used to refine prognostication in AML and to inform therapeutic approaches. We also review the current challenges in translating genomic studies to the clinical setting, which remains a significant challenge and an urgent priority.

Free full text available from *Hematology*
PMID: 23233557

D) Genetic Profiling in Acute Myeloid Leukaemia--Where Are We and What is Its Role in Patient Management

Ofran Y, Rowe JM. Genetic profiling in acute myeloid leukaemia--where are we and what is its role in patient management. *Br J Haematol.* 2013 Feb; 160(3): 303-320.

Summary: Genetic profiling in acute myeloid leukaemia (AML) is a moving target. Only 4 years ago, AML was re-classified, based on karyotypic abnormalities. However, numerous important new mutations and other genetic abnormalities that were not considered in this classification have been identified. Current cytogenetic-based classification is limited by the substantial number of intermediate-risk patients in whom the preferred therapy is debatable. In addition, the majority of AML patients co-express multiple mutations and cannot be easily categorized into predefined homogenous groups. The tremendous progress in mass sequencing allows parallel identification of multiple genetic aberrations in large cohorts. Thus, a new concept of genetic profiling has arisen. Genes and proteins biologically interact with each other; therefore, it should not be surprising that mutations in different genes interact. Prognosis is determined by the composition of mutations and aberrations in leukaemic stem cells. As a consequence, clinical decisions no longer rely on scant genetic data and require comprehensive genetic evaluation. Some non-genetic

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parameters are also important and should be incorporated into the clinical decision algorithm. Genetic interaction-based profiles are challenging and recent studies demonstrate an improvement in prognostic predictions with this model. Thus, genetic profiling is likely to have a major therapeutic impact, at least for intermediate-risk cytogenetics.

Full text available from *British Journal of Haematology* (subscription required)
PMID: 23240632

E) Prognostic Significance of the European LeukemiaNet Standardized System for Reporting Cytogenetic and Molecular Alterations in Adults with Acute Myeloid Leukemia

Mrózek K, Marcucci G, Nicolet D, et al. Prognostic significance of the European LeukemiaNet standardized system for reporting cytogenetic and molecular alterations in adults with acute myeloid leukemia. *J Clin Oncol.* 2012 Dec 20; 30(36): 4515-4523.

Summary: To evaluate the prognostic significance of the international European LeukemiaNet (ELN) guidelines for reporting genetic alterations in acute myeloid leukemia (AML). PATIENTS AND METHODS: We analyzed 1,550 adults with primary AML, treated on Cancer and Leukemia Group B first-line trials, who had pretreatment cytogenetics and, for cytogenetically normal patients, mutational status of NPM1, CEBPA, and FLT3 available. We compared complete remission (CR) rates, disease-free survival (DFS), and overall survival (OS) among patients classified into the four ELN genetic groups (favorable, intermediate-I, intermediate-II, adverse) separately for 818 younger (age < 60 years) and 732 older (age >/= 60 years) patients. RESULTS: The percentages of younger versus older patients in the favorable (41% v 20%; P < .001), intermediate-II (19% v 30%; P < .001), and adverse (22% v 31%; P < .001) genetic groups differed. The favorable group had the best and the adverse group the worst CR rates, DFS, and OS in both age groups. Both intermediate groups had significantly worse outcomes than the favorable but better than the adverse group. Intermediate-I and intermediate-II groups in older patients had similar outcomes, whereas the intermediate-II group in younger patients had better OS but not better CR rates or DFS than

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the intermediate-I group. The prognostic significance of ELN classification was confirmed by multivariable analyses. For each ELN group, older patients had worse outcomes than younger patients. CONCLUSION: The ELN classification clearly separates the genetic groups by outcome, supporting its use for risk stratification in clinical trials. Because they have different proportions of genetic alterations and outcomes, younger and older patients should be reported separately when using the ELN classification.

Full text available *Journal of Clinical Oncology* (subscription required)
PMID: 22987078

F) Mutational Landscape of AML with Normal Cytogenetics: Biological and Clinical Implications

Martelli MP, Sportoletti P, Tiacci E, Martelli MF, Falini B. Mutational landscape of AML with normal cytogenetics: biological and clinical implications. *Blood Rev.* 2013 Jan; 27(1): 13-22.

Summary: Acute myeloid leukemia (AML) is a molecularly heterogeneous disease. Based on cytogenetics and FISH, AML patients are stratified into three major risk categories: favourable, intermediate and unfavourable. However, prognostic stratification and treatment decision for the intermediate risk category, that mostly comprises AML patients with normal cytogenetics (CN-AML), has been difficult due to the clinical heterogeneity and scarce knowledge of the molecular alterations underlying this large AML subgroup. During the past decade, the identification of several mutations associated with CN-AML has resulted into important advances in the AML field. In this review, we address the biological features of the main mutations associated with CN-AML and the impact of next generation sequencing studies in expanding our knowledge of the molecular landscape of CN-AML. In addition, we outline the prognostic value of mutations for risk stratification of CN-AML patients and discuss the potential of mutations discovery process for developing new molecular targeted therapies.

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PMID: 23261068

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G) Prospective Evaluation of Gene Mutations and Minimal Residual Disease in Patients with Core Binding Factor Acute Myeloid Leukemia

Jourdan E, Boissel N, Chevret S. Prospective evaluation of gene mutations and minimal residual disease in patients with core binding factor acute myeloid leukemia. *Blood.* 2013 Mar 21; 121(12): 2213-2223.

Summary: Not all patients with core binding factor acute myeloid leukemia (CBF-AML) display a good outcome. Modern risk factors include KIT and/or FLT3 gene mutations and minimal residual disease (MRD) levels, but their respective values have never been prospectively assessed. A total of 198 CBF-AML patients were randomized between a reinforced and a standard induction course, followed by 3 high-dose cytarabine consolidation courses. MRD levels were monitored prospectively. Gene mutations were screened at diagnosis. Despite a more rapid MRD decrease after reinforced induction, induction arm did not influence relapse-free survival (RFS) (64% in both arms; P = .91). Higher WBC, KIT, and/or FLT3-ITD/TKD gene mutations, and a less than 3-log MRD reduction after first consolidation, were associated with a higher specific hazard of relapse, but MRD remained the sole prognostic factor in multivariate analysis. At 36 months, cumulative incidence of relapse and RFS were 22% vs 54% (P < .001) and 73% vs 44% (P < .001) in patients who achieved 3-log MRD reduction vs the others. These results suggest that MRD, rather than gene mutations, should be used for future treatment stratifications in CBF-AML patients. This trial was registered at EudraCT as #2006-005163-26 and at www.clinicaltrials.gov as #NCT 00428558.

Free full text available from *Blood*
PMID: 23321257

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3.8.2 Lymphoid Neoplasia

3.8.2.1 Precursor Lymphoid Neoplasms

A) Immunologic Minimal Residual Disease Detection in Acute Lymphoblastic Leukemia: A Comparative Approach to Molecular Testing

Coustan-Smith E, Campana D. Immunologic minimal residual disease detection in acute lymphoblastic leukemia: a comparative approach to molecular testing. *Best Pract Res Clin Haematol*. 2010 Sep;23(3):347-58.

Summary: The generation of antisera directed against leukocyte differentiation antigens opened the possibility of studying minimal residual disease (MRD) in patients with acute lymphoblastic leukemia (ALL). During the three decades that followed the pioneering studies in this field, great progress has been made in the development of a wide array of monoclonal antibodies and of flow cytometric techniques for rare event detection. This advance was accompanied by an increasingly greater understanding of the immunophenotypic features of leukemic and normal lymphoid cells, and of the antigenic differences that make MRD studies possible. In parallel, molecular methods for MRD detection were established. The systematic application of immunologic and molecular techniques to study MRD in clinical samples has demonstrated the clinical significance of MRD in patients, leading to the use of MRD to regulate treatment intensity in many contemporary protocols. In this article, we discuss methodologic issues related to the immunologic monitoring of MRD and the evidence supporting its clinical significance, and compare the advantages and limitations of this approach to those of molecular monitoring of MRD.

Free full text available from *Best Practice & Research Clinical Haematology*

PMID: 21112034

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3.8.2.2 Mature B-cell Neoplasms

A) B Cells Behaving Badly: A Better Basis to Behold Belligerence in B-cell Lymphomas

Bagg A. B cells behaving badly: a better basis to behold belligerence in B-cell lymphomas. *Hematology* Am Soc Hematol Educ Program. 2011;2011:330-5.

Summary: A plethora of genetic abnormalities has been described in B-cell lymphomas, some of which arise when physiologic mechanisms involved in the generation of immunologic diversity go awry. Several different lymphoma types, such as follicular lymphoma (FL), mantle cell lymphoma (MCL), and Burkitt lymphoma (BL), are associated with hallmark translocations that occur as a consequence of these errors (t(14;18)(q32;q21), t(11;14)(q13;q32), and t(8;14)(q24;q32), respectively); however, none of these associations is absolute and none is completely diagnostically specific or sensitive. The advantages and limitations of a variety of different testing strategies in the 2 most common lymphomas, FL and diffuse large B-cell lymphoma (DLBCL), are reviewed herein, including an evaluation of the role of PCR-based approaches, FISH, and more nascent genomic technologies. The use of immunophenotypic strategies that may potentially provide, albeit imperfectly, more user-friendly surrogates for underlying genetic aberrations and cell-of-origin designations derived from gene-expression profiling analyses are also discussed. Finally, a newly designated category of lymphoma with features intermediate between DLBCL and BL is appraised, highlighting the central role of genetic analysis in this diagnostic gray zone.

Free full text available from *Hematology*
PMID: 22160054

B) Significantly Improved PCR-based Clonality Testing in B-cell Malignancies by Use of Multiple Immunoglobulin Gene Targets. Report of the BIOMED-2 Concerted Action BHMS-CT98-3936

Evans PA, Pott CH, Groenen PJ, et al. Significantly improved PCR-based clonality testing in B-cell malignancies by the use of multiple immunoglobulin gene targets. Report of the BIOMED-2 concerted action BHMS-CT98-3936. *Leukemia.* 2007 Feb;21(2):207-14.

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Summary: Polymerase chain reaction (PCR) assessment of clonal immunoglobulin (Ig) and T-cell receptor (TCR) gene rearrangements is an important diagnostic tool in mature B-cell neoplasms. However, lack of standardized PCR protocols resulting in a high level of false negativity has hampered comparability of data in previous clonality studies. In order to address these problems, 22 European laboratories investigated the Ig/TCR rearrangement patterns as well as t(14;18) and t(11;14) translocations of 369 B-cell malignancies belonging to five WHO-defined entities using the standardized BIOMED-2 multiplex PCR tubes accompanied by international pathology panel review. B-cell clonality was detected by combined use of the IGH and IGK multiplex PCR assays in all 260 definitive cases of B-cell chronic lymphocytic leukemia (n=56), mantle cell lymphoma (n=54), marginal zone lymphoma (n=41) and follicular lymphoma (n=109). Two of 109 cases of diffuse large B-cell lymphoma showed no detectable clonal marker. The use of these techniques to assign cell lineage should be treated with caution as additional clonal TCR gene rearrangements were frequently detected in all disease categories. Our study indicates that the BIOMED-2 multiplex PCR assays provide a powerful strategy for clonality assessment in B-cell malignancies resulting in high Ig clonality detection rates particularly when IGH and IGK strategies are combined.

Free full text available from *Leukemia*
PMID: 17170731

C) Bone Marrow Trephines Containing Lymphoid Aggregates from Patients with Rheumatoid and Other Autoimmune Disorders Frequently Show Clonal B-cell Infiltrates

Engels K, Oeschger S, Hansmann ML, Hillebrand M, Kriener S. Bone marrow trephines containing lymphoid aggregates from patients with rheumatoid and other autoimmune disorders frequently show clonal B-cell infiltrates. *Hum Pathol.* 2007 Sep; 38(9): 1402-1411.

Summary: In bone marrow trephines, morphological and immunohistochemical criteria may not be sufficient to discriminate reactive from malignant lymphoid infiltrates. The aim of this study was to determine whether the detection of clonal immunoglobulin heavy

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chain (IGH) gene rearrangements is a reliable and specific marker for malignant B-cell clones in bone marrow biopsies. Bone marrow trephines with infiltration by different types of low-grade B-cell non-Hodgkin lymphoma (n = 32), reactive lymphoid hyperplasia (n = 18), and reactive lymphoid aggregates (n = 15), including 5 patients with rheumatoid or other autoimmune disorders, were analyzed by morphology, immunohistochemistry, IGH gene rearrangement (polymerase chain reaction), and DNA sequence analysis in selected cases. In 22 (68.8%) of 32 patients with B-cell non-Hodgkin lymphoma, a clonal IGH gene rearrangement was detected. Of the reactive cases, 1 of 18 patients with lymphoid hyperplasia demonstrated clonality, and 9 (60%) of 15 patients with reactive lymphoid aggregates gave a clonal result (GeneScan analysis). DNA sequence analysis was performed in 7 of the latter patients confirming clonality in 6. Four of the patients with B-cell clonality had an autoimmune disorder. None of these patients developed a malignant lymphoma during follow-up. Thus, the molecular detection of a clonal rearrangement of the IGH gene may support the diagnosis of a malignant lymphoma infiltrating the bone marrow. However, morphologically and immunohistochemically benign lymphoid aggregates might also harbor B-cell clones especially in patients with autoimmune disorders. Therefore, the detection of clonality has to be interpreted with utmost care and does not qualify for the unequivocal diagnosis of a malignant B-cell lymphoma.

Full text available from *Human Pathology* (USD 31.50)
PMID: 17560629

D) BIOMED-2 PCR Assays for IGK Gene Rearrangements Are Essential for B-cell Clonality Analysis in Follicular Lymphoma

Payne K, Wright P, Grant JW, et al. BIOMED-2 PCR assays for IGK gene rearrangements are essential for B-cell clonality analysis in follicular lymphoma. *Br J Haematol.* 2011 Oct; 155(1): 84-92.

Summary: B-cell clonality analysis is commonly performed by polymerase chain reaction (PCR) targeting the IGH genes although a high false-negative rate is recognized for germinal centre/post-germinal centre B-cell malignancies, especially follicular lymphoma. We assessed the diagnostic value of BIOMED-2 IGK assays and

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investigated the cause of IGH PCR failure in 77 patients with follicular lymphoma. Using the full set of BIOMED-2 reactions, clonal immunoglobulin gene rearrangements were detected in 74 (96%) cases. The clonality detection rate was 86% by two IGK reactions but only 68% by five IGH reactions ($P < 0.001$). Sequencing of the clonal PCR products showed significantly fewer somatic mutations in the rearranged IGKV (9/27 cases, 33%, mean mutation rate 0.5%) than IGHV (17/17 cases, 100%, rate 11.0%) ($P < 0.01$). All IGHV-IGHJ PCR failures occurred in cases with at least one mutation at the corresponding IGHV primer binding sites. t(14:18)(q32:q21)/IGH-BCL2 was detected in 50 of 71 (70%) cases and the presence of the translocation was not associated with the poor performance of IGH assays. Our results showed that BIOMED-2 IGK assays are significantly more sensitive than IGH assays in follicular lymphoma due to the fact that the rearranged IGKV is less frequently targeted by somatic hypermutation than IGHV, and therefore, are essential in routine clonality analysis of these lymphomas.

Free full text available from *British Journal of Haematology*
PMID: 21790530

E) The Basis and Rational Use of Molecular Genetic Testing in Mature B-cell Lymphomas

Roullet M, Bagg A. The basis and rational use of molecular genetic testing in mature B-cell lymphomas. *Adv Anat Pathol.* 2010 Sep; 17(5): 333-358.

Summary: An increasing number of neoplasms are associated with variably specific genetic abnormalities. This is best exemplified by hematological malignancies, in which there is a growing list of entities that are defined by their genetic lesion(s); this is not (yet) the case in mature B-cell lymphomas. However, enhanced insights into the pathogenesis of this large and diverse group of lymphomas have emerged with the ongoing unraveling of a plethora of fascinating genetic abnormalities. The purpose of this review is to synthesize well-recognized data and nascent discoveries in our understanding of the genetic basis of a spectrum of mature B-cell lymphomas, and how this may be applied to contemporary clinical practice. Despite the explosion of new and exciting knowledge in this arena, with the

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potential for enhanced diagnostic and prognostic strategies, it is essential to remain cognizant of the limitations (and complexity) of genetic investigations, so that assays can be developed and used both judiciously and rationally.

Full text available from *Advances in Anatomic Pathology* (subscription required)
PMID: 20733353

F) Both Variant and IGHV4-34-Expressing Hairy Cell Leukemia Lack the BRAF V600E Mutation

Xi L, Arons E, Navarro W, et al. Both variant and IGHV4-34-expressing hairy cell leukemia lack the BRAF V600E mutation. *Blood.* 2012 Apr 5; 119(14): 3330-3332.

Summary: Recently, the BRAF V600E mutation was reported in all cases of hairy cell leukemia (HCL) but not in other peripheral B-cell neoplasms. We wished to confirm these results and assess BRAF status in well-characterized cases of HCL associated with poor prognosis, including the immunophenotypically defined HCL variant (HCLv) and HCL expressing the IGHV4-34 immunoglobulin rearrangement. Fifty-three classic HCL (HCLc) and 16 HCLv cases were analyzed for BRAF, including 5 HCLc and 8 HCLv expressing IGHV4-34. BRAF was mutated in 42 (79%) HCLc, but wild-type in 11 (21%) HCLc and 16 (100%) HCLv. All 13 IGHV4-34(+) HCLs were wild-type. IGHV gene usage in the 11 HCLc BRAF wild-type cases included 5 IGHV4-34, 5 other, and 1 unknown. Our results suggest that HCLv and IGHV4-34(+) HCLs have a different pathogenesis than HCLc and that a significant minority of other HCLc are also wild-type for BRAF V600.

Free full text available from PubMed
PMID: 22210875

G) Malleable Immunoglobulin Genes and Hematopathology - The Good, the Bad, and the Ugly: A Paper from the 2007 William Beaumont Hospital Symposium on Molecular Pathology

Bagg A. Malleable immunoglobulin genes and hematopathology - the good, the bad, and the ugly: a paper from the 2007 William Beaumont

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hospital symposium on molecular pathology. *J Mol Diagn.* 2008 Sep; 10(5): 396-410.

Summary: Immunoglobulin gene rearrangement analysis is one of the more commonly performed assays available on the hematopathology menu of clinical molecular pathology laboratories. The analysis of these rearrangements provides useful information on a number of different levels in the evaluation of lymphoproliferations. An appreciation of the various mechanisms involved in the numerous physiological pathways affecting the immunoglobulin genes, and hence antibody molecules, is central to an understanding of B-cell development vis-a-vis the generation of immunological diversity. Knowledge about the intricate complexities of these mechanisms is also germane to an evaluation of testing methodologies. With this information, it is easier to develop an understanding of how contemporary molecular testing of immunoglobulin gene rearrangements has evolved, from historically quite heterogeneous, fairly flawed, and rather ugly approaches to current more-standardized protocols. In addition, recognition of how such genetic changes with good intentions can turn bad has fostered increasing insights into the pathogenesis of B-cell lymphomas and leukemias. Despite the significant improvements in the design of immunoglobulin gene rearrangement assays, numerous pitfalls and caveats remain. Accordingly, it is crucial to consider such testing a tool, and although most useful, it is one of many tools that may be required to build cogent diagnoses.

Free full text available from PubMed
PMID: 18687793

H) Genomic Aberrations and Survival in Chronic Lymphocytic Leukemia

Döhner H, Stilgenbauer S, Benner A, et al. Genomic Aberrations and Survival in Chronic Lymphocytic Leukemia. *N Engl J Med* 2000; 343:1910-1916

Summary: Fluorescence in situ hybridization has improved the detection of genomic aberrations in chronic lymphocytic leukemia. We used this method to identify chromosomal abnormalities in patients

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with chronic lymphocytic leukemia and assessed their prognostic implications. Methods--Mononuclear cells from the blood of 325 patients with chronic lymphocytic leukemia were analyzed by fluorescence in situ hybridization for deletions in chromosome bands 6q21, 11q22–23, 13q14, and 17p13; trisomy of bands 3q26, 8q24, and 12q13; and translocations involving band 14q32. Molecular cytogenetic data were correlated with clinical findings. Results—Chromosomal aberrations were detected in 268 of 325 cases (82 percent). The most frequent changes were a deletion in 13q (55 percent), a deletion in 11q (18 percent), trisomy of 12q (16 percent), a deletion in 17p (7 percent), and a deletion in 6q (6 percent). Five categories were defined with a statistical model: 17p deletion, 11q deletion, 12q trisomy, normal karyotype, and 13q deletion as the sole abnormality; the median survival times for patients in these groups were 32, 79, 114, 111, and 133 months, respectively. Patients in the 17p- and 11q-deletion groups had more advanced disease than those in the other three groups. Patients with 17p deletions had the shortest median treatment-free interval (9 months), and those with 13q deletions had the longest (92 months). In multivariate analysis, the presence or absence of a 17p deletion, the presence or absence of an 11q deletion, age, Binet stage, the serum lactate dehydrogenase level, and the white-cell count gave significant prognostic information. Conclusions--Genomic aberrations in chronic lymphocytic leukemia are important independent predictors of disease progression and survival. These findings have implications for the design of risk-adapted treatment strategies.

Free full article available from *New England Journal of Medicine*
PMID: 11136261

I) Chronic Lymphocytic Leukemia: A Clinical and Molecular Heterogenous Disease

Rodríguez-Vicente AE, Díaz MG, Hernández-Rivas JM. Chronic lymphocytic leukemia: a clinical and molecular heterogenous disease. *Cancer Genet.* 2013 Mar; 206(3): 49-62.

Summary: The clinical heterogeneity that characterizes chronic lymphocytic leukemia (CLL), with survival times ranging from months to decades, reflects its biological diversity. Our understanding of the

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biology of CLL has helped us identify several markers of prognostic significance, by which CLL can be differentiated into several distinct diseases. The presence of specific chromosomal abnormalities is a prognostic indicator of disease progression and survival. Conventional cytogenetic analyses have revealed chromosomal aberrations in 40-50% of patients, but the detection of abnormalities is limited by the low mitotic activity of CLL cells. Metaphase analysis has recently undergone a "revival" because the metaphase yield has been improved by stimulation of CLL cells with alternative methods. Fluorescence in situ hybridization identifies chromosomal changes in approximately 80% of patients with CLL, and comparative genomic hybridization using high-density arrays (i.e., array comparative genomic hybridization [aCGH]) enables high-resolution genome-wide scanning for detecting copy number alterations in a single hybridization. The mutational status of the immunoglobulin heavy chain variable (IGHV) genes identifies two subsets of CLL with different outcomes. Unfortunately, the determination of IGHV mutation status may not be practical in all laboratories, and for this reason characteristics that are correlated with IGHV mutation status are needed-zeta-chain associated (TCR) protein kinase 70 kDa (ZAP-70) being that most commonly used currently in routine clinical practice. Whole genome sequencing has offered new insights into the mutational status of the disease, highlighting the role of several genes previously unrelated to CLL. Of these, NOTCH1 and SF3B1 are the most frequently mutated genes that predict poor prognosis. MicroRNA alterations are also involved in the initiation and progression of CLL, and the expression levels of some microRNAs correlate with previously established prognostic markers such as IGHV mutation status or ZAP-70. In addition, both global and gene-specific aberrant DNA methylation have been observed in CLL. Aberrant methylation has been described for genes that are specifically deregulated in CLL, such as BCL2, TCL1, and ZAP-70. Expanding knowledge of aberrant methylation profiles in CLL has a potential future impact on diagnosis, prognosis, and prediction of treatment response in CLL patients.

Full text available from *Cancer Genetics* (USD 31.50)
PMID: 23531595

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J) Clinical Laboratory Analysis of Immunoglobulin Heavy Chain Variable Region Genes for Chronic Lymphocytic Leukemia Prognosis

Szankasi P, Bahler DW. Clinical laboratory analysis of immunoglobulin heavy chain variable region genes for chronic lymphocytic leukemia prognosis. *J Mol Diagn.* 2010 Mar; 12(2): 244-249.

Summary: Chronic lymphocytic leukemia (CLL) is the most common leukemia affecting adults in the western world. The clinical course of CLL is highly variable: cases that express mutated immunoglobulin heavy chain variable regions (IgV(H)) typically have a more indolent clinical course compared with those with unmutated IgV(H). The use of the V(H)3-21 variable region has also been found to confer a poor prognosis, independent of mutation status. Here we describe an assay for the identification of the expressed V(H) segment and its mutation status in CLL. This test uses whole blood-derived RNA and PCR primers annealing to the leader regions and the joining region segments. This approach allows more accurate determination of the IgV(H) mutation status relative to using framework region specific V(H) primers. An additional primer specific for the leader region of the V(H)3-21 segment is described and is shown to be necessary to identify this diagnostically important variable region. We successfully analyzed 99 of 103 samples, including five expressing the V(H)3-21 variable region. Approximately 5% of cases had complement determining region 3 sequences similar to previously reported cases, and overrepresentation of the V(H)1-69 segment was observed among unmutated cases. These results confirm the proper functioning and high success rate of this valuable prognostic for CLL designed for the use in a clinical laboratory setting.

Free full text available from PubMed
PMID: 20110453

K) Implications of New Prognostic Markers in Chronic Lymphocytic Leukemia

Chiorazzi N. Implications of new prognostic markers in chronic lymphocytic leukemia. *Hematology Am Soc Hematol Educ Program.* 2012 2012:76-87.

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Summary: Several prognostic markers based on genetic, phenotypic, and molecular characteristics of chronic lymphocytic leukemia (CLL) B cells have emerged in the past decade. The clinical utility of these newer prognostic indicators, alone or in combination with each other and other clinical predictive systems, is still being determined. This chapter attempts to define biologic and molecular underpinnings of 3 sets of prognostic indicators in CLL: genetic abnormalities quantified by FISH and/or defined by exploratory sensitive molecular techniques, expression of specific proteins in or on CLL cells (ie, CD38, CD49d, and ZAP-70), and the IGHV mutation status of a CLL clone. Although not demonstrated conclusively, each probably reflects the biologic properties of the leukemic cells of individual CLL patients. This reflection may be direct, indicating a specific property of the CLL cell itself, or indirect, representing how the CLL cell interacts with the host's microenvironment. The new tyrosine kinase inhibitors that are currently in clinical trials support this interpretation. These and other biology-based indicators of patient clinical course and outcome can be used as starting points from which to understand and treat CLL.

Free full text available from *Hematology*
PMID: 23233564

3.8.2.3 Mature T-cell Neoplasms

A) Powerful Strategy for Polymerase Chain Reaction-Based Clonality Assessment in T-Cell Malignancies Report of the BIOMED-2 Concerted Action BHM4 CT98-3936

Brüggemann M, White H, Gaulard P, et al. Powerful strategy for polymerase chain reaction-based clonality assessment in T-cell malignancies. Report of the BIOMED-2 Concerted Action BHM4 CT98-3936. *Leukemia.* 2007 Feb;21:215-221.

Summary: Polymerase chain reaction (PCR) assessment of clonal T-cell receptor (TCR) and immunoglobulin (Ig) gene rearrangements is an important diagnostic tool in mature T-cell neoplasms. However, lack of standardized primers and PCR protocols has hampered comparability of data in previous clonality studies. To obtain reference

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values for Ig/TCR rearrangement patterns, 19 European laboratories investigated 188 T-cell malignancies belonging to five World Health Organization-defined entities. The TCR/Ig spectrum of each sample was analyzed in duplicate in two different laboratories using the standardized BIOMED-2 PCR multiplex tubes accompanied by international pathology panel review. TCR clonality was detected in 99% (143/145) of all definite cases of T-cell prolymphocytic leukemia, T-cell large granular lymphocytic leukemia, peripheral T-cell lymphoma (unspecified) and angioimmunoblastic T-cell lymphoma (AILT), whereas nine of 43 anaplastic large cell lymphomas did not show clonal TCR rearrangements. Combined use of TCRB and TCRG genes revealed two or more clonal signals in 95% of all TCR clonal cases. Ig clonality was mostly restricted to AILT. Our study indicates that the BIOMED-2 multiplex PCR tubes provide a powerful strategy for clonality assessment in T-cell malignancies assisting the firm diagnosis of T-cell neoplasms. The detected TCR gene rearrangements can also be used as PCR targets for monitoring of minimal residual disease.

Free full text available from *Leukemia*
PMID: 17170730

B) The Indicative Effect of Notch1 Expression for the Prognosis of T-cell Acute Lymphocytic Leukemia: A Systematic Review
Ma J, Wu M. The indicative effect of Notch1 expression for the prognosis of T-cell acute lymphocytic leukemia: a systematic review. *Mol Biol Rep.* 2012 May; 39(5): 6095-6100.

Summary: To explore the relationship of Notch1 mutation in T-ALL with the survival rate of T-ALL patients. The PubMed database, the Cochrane Library, conference proceedings, EMBASE databases, and references of published trials and review articles were searched. Two reviewers independently assessed the quality of the trials and extracted data. Hazard ratios (HRs) for event-free survival (EFS) were pooled by STATA package. Seven trials involving 964 patients with T-ALL were ultimately analyzed. Seven hundred and eleven patients were children (age <18 years), 253 patients were adults (age >/=18 years). The pooled HR showed that Notch1 mutated group could not prolong EFS than Notch1 WT group both in children and adult

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patients. Although constitutively activated forms of the NOTCH1 receptor are potent inducers of T-ALL, our results suggest that Notch1 mutation could not become an indicator for EFS in T-ALL.

Full text available from *Molecular Biology Reports* (USD 39.95)
PMID: 22311010

C) EuroClonality/BIOMED-2 Guidelines for Interpretation and Reporting of Ig/TCR Clonality Testing in Suspected Lymphoproliferations

Langerak AW, Groenen PJ, Brüggemann M, et al. EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations. *Leukemia.* 2012 Oct; 26(10): 2159-2171.

Summary: PCR-based immunoglobulin (Ig)/T-cell receptor (TCR) clonality testing in suspected lymphoproliferations has largely been standardized and has consequently become technically feasible in a routine diagnostic setting. Standardization of the pre-analytical and post-analytical phases is now essential to prevent misinterpretation and incorrect conclusions derived from clonality data. As clonality testing is not a quantitative assay, but rather concerns recognition of molecular patterns, guidelines for reliable interpretation and reporting are mandatory. Here, the EuroClonality (BIOMED-2) consortium summarizes important pre- and post-analytical aspects of clonality testing, provides guidelines for interpretation of clonality testing results, and presents a uniform way to report the results of the Ig/TCR assays. Starting from an immunobiological concept, two levels to report Ig/TCR profiles are discerned: the technical description of individual (multiplex) PCR reactions and the overall molecular conclusion for B and T cells. Collectively, the EuroClonality (BIOMED-2) guidelines and consensus reporting system should help to improve the general performance level of clonality assessment and interpretation, which will directly impact on routine clinical management (standardized best-practice) in patients with suspected lymphoproliferations.

Free full text available from PubMed
PMID: 22918122

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D) The Presence of Dominant T-cell Clones in Peripheral Blood of Patients with Collagen Vascular Disorders: A Prospective Study of 97 Cases

Dereure O, Gubler B, Bessis D, et al. The presence of dominant T-cell clones in peripheral blood of patients with collagen vascular disorders: a prospective study of 97 cases. *Br J Dermatol.* 2006 Mar; 154(3): 445-449.

Summary: T-lymphocyte dysfunction has been seldom investigated in collagen vascular disorders. The search for dominant T-cell clones has been scarcely reported, although the presence of such clones might be expected in disorders showing immune responses directed against a variety of autoantigens. OBJECTIVES: We conducted a systematic search for dominant T-cell clones in peripheral blood in patients with collagen vascular disorders. Patients and methods Ninety-seven patients with collagen vascular disorders were studied (7 cutaneous and 38 systemic lupus erythematosus; 8 multiple morphea; 12 regional scleroderma; 32 systemic sclerosis of the CREST type). A dominant T-cell clone was searched for in peripheral blood by polymerase chain reaction targeting the T-cell receptor gamma chain followed by a size analysis of amplified fragments. Peripheral blood from patients with nonlymphocyte-dependent disorders and matched by age and sex was assessed in the same conditions. Results in both groups were compared using nonparametric statistical tests. RESULTS: Overall, a circulating dominant T-cell clone was found in 52% of patients compared with 16.9% in controls. More precisely, such a dominant clone was present in 43% and 37% of cutaneous and systemic lupus erythematosus, respectively, in 75% of multiple morphea, 75% of regional scleroderma and 60% of CREST syndrome patients. The percentages in all subsets of patients were significantly higher than in the control group. CONCLUSIONS: The presence of a dominant T-cell clone in peripheral blood is significantly more frequent in collagen vascular disorders than in controls, especially in patients with scleroderma, whatever the clinical subset, which suggests T-cell involvement in the immune response dysfunction in these diseases classically characterized by disturbances of B lymphocytes. The relevance of such a dominant clone regarding diagnosis, pathomechanisms, long-

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term outcome and visceral prognosis of these diseases as well as therapeutic decisions remains to be evaluated.

Full text available from *British Journal of Dermatology* (subscription required)
PMID: 16445773

E) Assay Design Affects the Interpretation of T-Cell Receptor Gamma Gene Rearrangements: Comparison of the Performance of a One-Tube Assay with the BIOMED-2-Based TCRG Gene Clonality Assay

Cushman-Vokoun AM, Connealy S, Greiner TC. Assay design affects the interpretation of T-cell receptor gamma gene rearrangements: comparison of the performance of a one-tube assay with the BIOMED-2-based TCRG gene clonality assay. *J Mol Diagn.* 2010 Nov; 12(6): 787-796.

Summary: Interpretation of capillary electrophoresis results derived from multiplexed fluorochrome-labeled primer sets can be complicated by small peaks, which may be incorrectly interpreted as clonal T-cell receptor-gamma gene rearrangements. In this report, different assay designs were used to illustrate how design may adversely affect specificity. Ten clinical cases, with subclonal peaks containing one of the two infrequently used joining genes, were identified with a tri-color, one-tube assay. The DNA was amplified with the same NED fluorochrome on all three joining primers, first combined (one-color assay) and then amplified separately using a single NED-labeled joining primer. The single primer assay design shows how insignificant peaks could easily be wrongly interpreted as clonal T-cell receptor-gamma gene rearrangements. Next, the performance of the one-tube assay was compared with the two-tube BIOMED-2-based TCRG Gene Clonality Assay in a series of 44 cases. Whereas sensitivity was similar between the two methods (92.9% vs. 96.4%; P = 0.55), specificity was significantly less in the BIOMED-2 assay (87.5% vs. 56.3%; P = 0.049) when a 2x ratio was used to define clonality. Specificity was improved to 81.3% by the use of a 5x peak height ratio (P = 0.626). These findings illustrate how extra caution is needed in interpreting a design with multiple, separate

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distributions, which is more difficult to interpret than a single distribution assay.

Free full text available from PubMed
PMID: 20959612

3.9 Lung Cancer

A) Advances in Treatment of Lung Cancer with Targeted Therapy
Cagle PT, Chirieac LR. Advances in treatment of lung cancer with targeted therapy. *Arch Pathol Lab Med*. 2012 May;136(5):504-509.

Summary: Ongoing preclinical investigations and clinical trials involving new targeted therapies promise to improve survival for patients with lung cancer. Targeted therapeutic agents, based on genetic mutations and signaling pathways altered in lung cancer, have added significantly to our armamentarium for lung cancer treatment while minimizing drug toxicity. To date, 4 targeted therapies have been approved for treatment of lung cancer by the US Food and Drug Administration: gefitinib in 2002, erlotinib in 2003, bevacizumab in 2006, and crizotinib in 2011. *Objective*.—To review targeted therapies in lung cancer, the molecular biomarkers that identify patients likely to benefit from these targeted therapies, the basic molecular biology principles, selected molecular diagnostic techniques, and pathologic features correlated with molecular abnormalities in lung cancer. To review new molecular abnormalities described in lung cancer that are predictive for response to novel promising targeted agents in various phases of clinical trials. *Data Sources*.—Review of the literature covering the molecular abnormalities of lung cancer with a focus on the molecular diagnostics and targeted therapy. Special emphasis is placed on summarizing evolving technologies useful in the diagnosis and characterization of lung cancer. *Conclusions*.—Molecular testing of lung cancer expands the expertise of the pathologist, who will identify the tumor markers that are predictive of sensitivity or resistance to various targeted therapies and allow patients with cancer to be selected for highly effective and less toxic therapies.

Article available from the CAP's *Archives*

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PMID: 22540298

B) Molecular Diagnosis of Lung Cancer

Iafrate J. PHC Webinar. Molecular diagnostics of lung cancer. [Webinar]. February 21, 2012. https://www1.gotomeeting.com/register/933180409. Accessed July 10, 2012.

Summary: Our knowledge of the genetic alterations that cause lung cancer has increased significantly over the past 5 years. This knowledge is now being exploited to develop targeted therapeutics with the potential for greater efficacy and fewer side effects. This webinar will review the current state of the molecular pathology of lung cancer, with both practical testing issues for the laboratory, and clinical implications of testing for oncology.

Archived webinar available for free

C) Molecular Testing Guideline for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors: Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology

Lindeman NI, Cagle PT, Beasley MB, et al. Molecular Testing Guideline for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors: Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. *Arch Pathol Lab Med.* 2013 Apr 3;

Summary: To establish evidence-based recommendations for the molecular analysis of lung cancers that are required to guide EGFR- and ALK-directed therapies, addressing which patients and samples should be tested, and when and how testing should be performed. Participants.-Three cochairs without conflicts of interest were selected, one from each of the 3 sponsoring professional societies: College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. Writing and advisory panels were constituted from additional experts

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from these societies. Evidence.-Three unbiased literature searches of electronic databases were performed to capture articles published from January 2004 through February 2012, yielding 1533 articles whose abstracts were screened to identify 521 pertinent articles that were then reviewed in detail for their relevance to the recommendations. Evidence was formally graded for each recommendation. Consensus Process.-Initial recommendations were formulated by the cochairs and panel members at a public meeting. Each guideline section was assigned to at least 2 panelists. Drafts were circulated to the writing panel (version 1), advisory panel (version 2), and the public (version 3) before submission (version 4). Conclusions.-The 37 guideline items address 14 subjects, including 15 recommendations (evidence grade A/B). The major recommendations are to use testing for EGFR mutations and ALK fusions to guide patient selection for therapy with an epidermal growth factor receptor (EGFR) or anaplastic lymphoma kinase (ALK) inhibitor, respectively, in all patients with advanced-stage adenocarcinoma, regardless of sex, race, smoking history, or other clinical risk factors, and to prioritize EGFR and ALK testing over other molecular predictive tests. As scientific discoveries and clinical practice outpace the completion of randomized clinical trials, evidence-based guidelines developed by expert practitioners are vital for communicating emerging clinical standards. Already, new treatments targeting genetic alterations in other, less common driver oncogenes are being evaluated in lung cancer, and testing for these may be addressed in future versions of these guidelines.

Free full text available from the CAP's *Archives*
PMID: 23551194

D) ROS1 Rearrangements Define a Unique Molecular Class of Lung Cancers

Bergethon K, Shaw AT, Ou SH, et al. ROS1 rearrangements define a unique molecular class of lung cancers. *J Clin Oncol.* 2012 Mar 10; 30(8): 863-870.

Summary: Chromosomal rearrangements involving the ROS1 receptor tyrosine kinase gene have recently been described in a subset of non-small-cell lung cancers (NSCLCs). Because little is

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known about these tumors, we examined the clinical characteristics and treatment outcomes of patients with NSCLC with ROS1 rearrangement. PATIENTS AND METHODS: Using a ROS1 fluorescent in situ hybridization (FISH) assay, we screened 1,073 patients with NSCLC and correlated ROS1 rearrangement status with clinical characteristics, overall survival, and when available, ALK rearrangement status. In vitro studies assessed the responsiveness of cells with ROS1 rearrangement to the tyrosine kinase inhibitor crizotinib. The clinical response of one patient with ROS1-rearranged NSCLC to crizotinib was investigated as part of an expanded phase I cohort. RESULTS: Of 1,073 tumors screened, 18 (1.7%) were ROS1 rearranged by FISH, and 31 (2.9%) were ALK rearranged. Compared with the ROS1-negative group, patients with ROS1 rearrangements were significantly younger and more likely to be never-smokers (each $P < .001$). All of the ROS1-positive tumors were adenocarcinomas, with a tendency toward higher grade. ROS1-positive and -negative groups showed no difference in overall survival. The HCC78 ROS1-rearranged NSCLC cell line and 293 cells transfected with CD74-ROS1 showed evidence of sensitivity to crizotinib. The patient treated with crizotinib showed tumor shrinkage, with a near complete response. CONCLUSION: ROS1 rearrangement defines a molecular subset of NSCLC with distinct clinical characteristics that are similar to those observed in patients with ALK-rearranged NSCLC. Crizotinib shows in vitro activity and early evidence of clinical activity in ROS1-rearranged NSCLC.

Free full text available from PubMed
PMID: 22215748

E) Response to Cabozantinib in Patients with RET Fusion-Positive Lung Adenocarcinomas

Drilon A, Wang L, Hasanovic A, et al. Response to Cabozantinib in Patients with RET Fusion-Positive Lung Adenocarcinomas. *Cancer Discov.* 2013 Mar 26: 630-5.

Summary: The discovery of RET fusions in lung cancers has uncovered a new therapeutic target for patients whose tumors harbor these changes. In an unselected population of non-small cell lung cancers (NSCLCs), RET fusions are present in 1-2% of cases. This

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incidence rises substantially, however, in never-smokers with lung adenocarcinomas that lack other known driver oncogenes. While pre-clinical data provide experimental support for the use of RET inhibitors in the treatment of RET fusion-positive tumors, clinical data on response are lacking. We report preliminary data for the first three patients treated with the RET inhibitor cabozantinib on a prospective phase 2 trial for patients with RET fusion-positive NSCLCs (NCT01639508). Confirmed partial responses were observed in two patients, including one harboring a novel TRIM33-RET fusion. A third patient with a KIF5B-RET fusion has had prolonged stable disease approaching 8 months (31 weeks). All three patients remain progression-free on treatment.

Full text available from *Cancer Discovery* (USD 35.00)
PMID: 23533264

3.10 Melanoma

3.10.1 Short Presentations in Emerging Concepts: Metastatic Melanoma

A) CAP Short Presentations in Emerging Concepts (SPECS): Emerging Concepts in Therapeutic Guidance for Metastatic Melanoma [PowerPoint slides]

College of American Pathologists. CAP Short Presentations in Emerging Concepts (SPECS): Emerging Concepts in Therapeutic Guidance for Metastatic Melanoma [PowerPoint slides]. Version 1.0.1. Northfield, IL: College of American Pathologists; 2012.

Access to slides

B) Improved Survival with Vemurafenib in Melanoma with BRAF V600E Mutation

Chapman PB, Hauschild A, Robert C, et al, Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med*. 2011; 364:2507-2516 June 30, 2011.

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Summary: Phase 1 and 2 clinical trials of the BRAF kinase inhibitor vemurafenib (PLX4032) have shown response rates of more than 50% in patients with metastatic melanoma with the BRAF V600E mutation. METHODS: We conducted a phase 3 randomized clinical trial comparing vemurafenib with dacarbazine in 675 patients with previously untreated, metastatic melanoma with the BRAF V600E mutation. Patients were randomly assigned to receive either vemurafenib (960 mg orally twice daily) or dacarbazine (1000 mg per square meter of body-surface area intravenously every 3 weeks). Coprimary end points were rates of overall and progression-free survival. Secondary end points included the response rate, response duration, and safety. A final analysis was planned after 196 deaths and an interim analysis after 98 deaths. RESULTS: At 6 months, overall survival was 84% (95% confidence interval [CI], 78 to 89) in the vemurafenib group and 64% (95% CI, 56 to 73) in the dacarbazine group. In the interim analysis for overall survival and final analysis for progression-free survival, vemurafenib was associated with a relative reduction of 63% in the risk of death and of 74% in the risk of either death or disease progression, as compared with dacarbazine ($P<0.001$ for both comparisons). After review of the interim analysis by an independent data and safety monitoring board, crossover from dacarbazine to vemurafenib was recommended. Response rates were 48% for vemurafenib and 5% for dacarbazine. Common adverse events associated with vemurafenib were arthralgia, rash, fatigue, alopecia, keratoacanthoma or squamous-cell carcinoma, photosensitivity, nausea, and diarrhea; 38% of patients required dose modification because of toxic effects. CONCLUSIONS: Vemurafenib produced improved rates of overall and progression-free survival in patients with previously untreated melanoma with the BRAF V600E mutation. (Funded by Hoffmann-La Roche; BRIM-3 ClinicalTrials.gov number, NCT01006980.).

Free full text available from *New England Journal of Medicine*
PMID: 21639808

C) Vemurafenib (Zelboraf®) Package Insert

Free full text available from FDA website

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3.10.2 Articles on Melanoma

A) Molecular Diagnostics in Melanoma: Current Status and Perspectives

Dadras SS. Molecular diagnostics in melanoma: current status and perspectives. *Arch Pathol Lab Med*. 2011 Jul; 135(7):860–9.

Summary: In the current “molecular” era, the advent of technology, such as array-based platforms, systems biology, and genome-wide approaches, has made it possible to examine human cancers, including melanoma, for genetic mutations, deletions, amplification, differentially regulated genes, and epigenetic changes. Advancement in current technologies is such that one can now examine ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and protein directly from the patient's own tumor. Objective.—To apply these new technologies in advancing molecular diagnostics in melanoma has historically suffered from a major obstacle, namely, the scarcity of fresh frozen, morphologically defined tumor banks, annotated with clinical information. Recently, some of the new platforms have advanced to permit utilization of formalin-fixed, paraffin-embedded (FFPE) tumor specimens as starting material. Data Sources.—This article reviews the latest technologies applied to FFPE melanoma sections, narrowing its focus on the utility of transcriptional profiling, especially for melastatin; comparative genomic hybridization; BRAF and NRAS mutational analysis; and micro ribonucleic acid profiling. Conclusion.—New molecular approaches are emerging and are likely to improve the classification of melanocytic neoplasms.

Free full text available from the CAP’s *Archives*
PMID: 21732775

B) Update on Fluorescence In Situ Hybridization in Melanoma: State of the Art

Gerami P, Zembowicz A. Update on fluorescence in situ hybridization in melanoma: state of the art. *Arch Pathol Lab Med*. 2011 July; 135(7):830–837.

Summary: Recent advances in understanding the molecular basis of melanoma have resulted in development of fluorescence in situ

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hybridization (FISH) protocols designed to detect genetic abnormalities discriminating melanoma from nevi. The most extensively studied is a 4-probe multicolor FISH probe panel targeting chromosomes 6 and 11. Validation studies showed promising sensitivity and specificity for distinguishing benign nevi and malignant melanoma by FISH. Recent studies show that a melanoma FISH assay has great potential for becoming an important diagnostic adjunct in classification of melanocytic lesions and in diagnosis of melanoma. Objective.—To present a comprehensive review of the science and practical aspects of FISH in melanoma for pathologists considering the use of melanoma FISH in their practice. Data Sources.—Review of the literature and personal experience of the authors. Conclusions.—Judicious use of a 4-probe multicolor melanoma FISH procedure can enhance accuracy for diagnosis of melanoma and improve classification of melanocytic proliferations.

Free full text available from the CAP's *Archives*
PMID: 21732770

C) New Strategies in Melanoma: Molecular Testing in Advanced Disease

Woodman SE, Lazar AJ, Aldape KD, Davies MA. New strategies in melanoma: molecular testing in advanced disease. *Clin Cancer Res.* 2012 Mar 1; 18(5): 1195-1200.

Summary: Melanoma is one of the most aggressive forms of skin cancer. The management of melanoma is evolving rapidly due to an improved understanding of the molecular heterogeneity of this disease and the development of effective, personalized, targeted therapy strategies. Although previous classification systems were based predominantly on clinical and histologic criteria, there is now a strong rationale for adding molecular markers to the diagnostic evaluation of these tumors. Research has shown that the types and prevalence of genetic alterations vary among melanoma subtypes. Thus, rational molecular testing should be based on an understanding of the events that are likely to occur in a given tumor and the clinical implications of test results. This review summarizes the existing data that support the rationale for molecular testing in clinically defined melanoma subtypes. Emerging challenges and controversies

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regarding the use of various molecular testing platforms, and their implications for clinical testing, are also discussed.

Free full text available from *Clinical Cancer Research*
PMID: 22275506

D) KIT as a Therapeutic Target in Metastatic Melanoma

Carvajal RD, Antonescu CR, Wolchok JD, et al. KIT as a therapeutic target in metastatic melanoma. *JAMA.* 2011 Jun 8; 305(22): 2327-2334.

Summary: Some melanomas arising from acral, mucosal, and chronically sun-damaged sites harbor activating mutations and amplification of the type III transmembrane receptor tyrosine kinase KIT. We explored the effects of KIT inhibition using imatinib mesylate in this molecular subset of disease. OBJECTIVE: To assess clinical effects of imatinib mesylate in patients with melanoma harboring KIT alterations. DESIGN, SETTING, AND PATIENTS: A single-group, open-label, phase 2 trial at 1 community and 5 academic oncology centers in the United States of 295 patients with melanoma screened for the presence of KIT mutations and amplification between April 23, 2007, and April 16, 2010. A total of 51 cases with such alterations were identified and 28 of these patients were treated who had advanced unresectable melanoma arising from acral, mucosal, and chronically sun-damaged sites. INTERVENTION: Imatinib mesylate, 400 mg orally twice daily. MAIN OUTCOME MEASURES: Radiographic response, with secondary end points including time to progression, overall survival, and correlation of molecular alterations and clinical response. RESULTS: Two complete responses lasting 94 (ongoing) and 95 weeks, 2 durable partial responses lasting 53 and 89 (ongoing) weeks, and 2 transient partial responses lasting 12 and 18 weeks among the 25 evaluable patients were observed. The overall durable response rate was 16% (95% confidence interval [CI], 2%-30%), with a median time to progression of 12 weeks (interquartile range [IQR], 6-18 weeks; 95% CI, 11-18 weeks), and a median overall survival of 46.3 weeks (IQR, 28 weeks-not achieved; 95% CI, 28 weeks-not achieved). Response rate was better in cases with mutations affecting recurrent hotspots or with a mutant to wild-type allelic ratio of more than 1 (40% vs 0%, P = .05), indicating positive

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selection for the mutated allele. CONCLUSIONS: Among patients with advanced melanoma harboring KIT alterations, treatment with imatinib mesylate results in significant clinical responses in a subset of patients. Responses may be limited to tumors harboring KIT alterations of proven functional relevance. Trial Registration clinicaltrials.gov Identifier: NCT00470470.

Free full text available from *Journal of the American Medical Association*
PMID: 21642685

3.11 Thyroid Cancer

3.11.1 Short Presentations in Emerging Concepts: Thyroid Cancer

A) CAP Short Presentations in Emerging Concepts (SPECS): Emerging Concepts in the Diagnosis and Work-up of Thyroid Cancer [PowerPoint slides]
College of American Pathologists. CAP Short Presentations in Emerging Concepts (SPECS): Emerging Concepts in the Diagnosis and Work-up of Thyroid Cancer [PowerPoint slides]. Version 1.0fc1. Northfield, IL: College of American Pathologists; 2012.

Access the slides

B) The Utility of BRAF Testing in the Management of Papillary Thyroid Cancer
Melck AL, Yip L, Carty SE. The utility of BRAF testing in the management of papillary thyroid cancer. *Oncologist.* 2010;15:1285-1293.

Summary: Over the last decade, investigators have developed a clearer understanding of the genetic alterations underlying thyroid carcinogenesis. A number of biomarkers involved in the pathogenesis of differentiated thyroid cancer have undergone intensive study, not only for their role in tumorigenesis, but also for their potential utility as

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diagnostic and prognostic indicators and therapeutic targets. This review summarizes the current literature surrounding BRAF and its significance in thyroid cancer. Further, we discuss how molecular analysis can be integrated into management algorithms for thyroid nodules and papillary thyroid cancer. We also review what is known, to date, about the association of BRAF and papillary microcarcinoma as well as using targeted therapies for BRAF as adjuvant treatment for metastatic papillary thyroid cancer.

Free full text available from *Oncologist*
PMID: 21147872

C) BRAF Mutations in Thyroid Tumors are Restricted to Papillary Carcinomas and Anaplastic or Poorly Differentiated Carcinomas Arising from Papillary Carcinomas

Nikiforova MN, Kimura ET, Gandhi M, et al. BRAF mutations in thyroid tumors are restricted to papillary carcinomas and anaplastic or poorly differentiated carcinomas arising from papillary carcinomas. *J Clin Endocrinol Metab* 2003;88:5399-5404

Summary: Activating point mutations of the BRAF gene have been recently reported in papillary thyroid carcinomas. In this study, we analyzed 320 thyroid tumors and six anaplastic carcinoma cell lines and detected BRAF mutations in 45 (38%) papillary carcinomas, two (13%) poorly-differentiated carcinomas, three (10%) anaplastic carcinomas, and five (83%) thyroid anaplastic carcinoma cell lines but not in follicular, Hürthle cell, and medullary carcinomas, follicular and Hürthle cell adenomas, or benign hyperplastic nodules. All mutations involved a T-->A transversion at nucleotide 1796. In papillary carcinomas, BRAF mutations were associated with older age, classic papillary carcinoma or tall cell variant histology, extrathyroidal extension, and more frequent presentation at stages III and IV. All BRAF-positive poorly differentiated and anaplastic carcinomas contained areas of preexisting papillary carcinoma, and mutation was present in both the well-differentiated and dedifferentiated components. These data indicate that BRAF mutations are restricted to papillary carcinomas and poorly differentiated and anaplastic carcinomas arising from papillary carcinomas. They are associated with distinct phenotypical and biological properties of papillary

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carcinomas and may participate in progression to poorly differentiated and anaplastic carcinomas.

Free full text available from *Journal of Clinical Endrocrinology and Metabolism*
PMID: 14602780

3.11.2 Articles on Thyroid Cancer

A) Molecular Diagnostics of Thyroid Tumors

Nikiforov YE. Molecular diagnostics of thyroid tumors. *Arch Pathol Lab Med.* 2011 May;
135(5):569–577.

Summary: Thyroid cancer is the most common type of endocrine malignancy and its incidence is steadily increasing. Papillary carcinoma and follicular carcinoma are the most common types of thyroid cancer and represent those tumor types for which use of molecular markers for diagnosis and prognostication is of high clinical significance. Objective.—To review the most common molecular alterations in thyroid cancer and their diagnostic and prognostic utility. Data Sources.—PubMed (US National Library of Medicine)–available review articles, peer-reviewed original articles, and experience of the author. Conclusions.—The most common molecular alterations in thyroid cancer include BRAF and RAS point mutations and RET/PTC and PAX8/PPARγ rearrangements. These nonoverlapping genetic alterations are found in more than 70% of papillary and follicular thyroid carcinomas. These molecular alterations can be detected in surgically resected samples and fine-needle aspiration samples from thyroid nodules and can be of significant diagnostic use. The diagnostic role of BRAF mutations has been studied most extensively, and recent studies also demonstrated a significant diagnostic utility of RAS, RET/PTC, and PAX8/PPARγ mutations, particularly in thyroid fine-needle aspiration samples with indeterminate cytology. In addition to the diagnostic use, BRAF V600E mutation can also be used for tumor prognostication, as this mutation is associated with higher rate of tumor recurrence and tumor-related mortality. The use of these and other emerging molecular markers is expected to improve significantly

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the accuracy of cancer diagnosis in thyroid nodules and allow more individualized surgical and postsurgical management of patients with thyroid cancer.

Free full text available from the CAP's *Archives*
PMID: 21526955

B) Association Between BRAF V600E Mutation and Mortality in Patients with Papillary Thyroid Cancer

Xing M, Alzahrani AS, Carson KA, et al. Association between BRAF V600E mutation and mortality in patients with papillary thyroid cancer. *JAMA.* 2013 Apr 10; 309(14): 1493-1501.

Summary: BRAF V600E is a prominent oncogene in papillary thyroid cancer (PTC), but its role in PTC-related patient mortality has not been established. OBJECTIVE: To investigate the relationship between BRAF V600E mutation and PTC-related mortality. DESIGN, SETTING, AND PARTICIPANTS: Retrospective study of 1849 patients (1411 women and 438 men) with a median age of 46 years (interquartile range, 34-58 years) and an overall median follow-up time of 33 months (interquartile range, 13-67 months) after initial treatment at 13 centers in 7 countries between 1978 and 2011. MAIN OUTCOMES AND MEASURES: Patient deaths specifically caused by PTC. RESULTS: Overall, mortality was 5.3% (45/845; 95% CI, 3.9%-7.1%) vs 1.1% (11/1004; 95% CI, 0.5%-2.0%) (P < .001) in BRAF V600E-positive vs mutation-negative patients. Deaths per 1000 person-years in the analysis of all PTC were 12.87 (95% CI, 9.61-17.24) vs 2.52 (95% CI, 1.40-4.55) in BRAF V600E-positive vs mutation-negative patients; the hazard ratio (HR) was 2.66 (95% CI, 1.30-5.43) after adjustment for age at diagnosis, sex, and medical center. Deaths per 1000 person-years in the analysis of the conventional variant of PTC were 11.80 (95% CI, 8.39-16.60) vs 2.25 (95% CI, 1.01-5.00) in BRAF V600E-positive vs mutation-negative patients; the adjusted HR was 3.53 (95% CI, 1.25-9.98). When lymph node metastasis, extrathyroidal invasion, and distant metastasis were also included in the model, the association of BRAF V600E with mortality for all PTC was no longer significant (HR, 1.21; 95% CI, 0.53-2.76). A higher BRAF V600E-associated patient mortality was also observed in several clinicopathological subcategories, but

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statistical significance was lost with adjustment for patient age, sex, and medical center. For example, in patients with lymph node metastasis, the deaths per 1000 person-years were 26.26 (95% CI, 19.18-35.94) vs 5.93 (95% CI, 2.96-11.86) in BRAF V600E-positive vs mutation-negative patients (unadjusted HR, 4.43 [95% CI, 2.06-9.51]; adjusted HR, 1.46 [95% CI, 0.62-3.47]). In patients with distant tumor metastasis, deaths per 1000 person-years were 87.72 (95% CI, 62.68-122.77) vs 32.28 (95% CI, 16.14-64.55) in BRAF V600E-positive vs mutation-negative patients (unadjusted HR, 2.63 [95% CI, 1.21-5.72]; adjusted HR, 0.84 [95% CI, 0.27-2.62]). CONCLUSIONS AND RELEVANCE: In this retrospective multicenter study, the presence of the BRAF V600E mutation was significantly associated with increased cancer-related mortality among patients with PTC. Because overall mortality in PTC is low and the association was not independent of tumor features, how to use BRAF V600E to manage mortality risk in patients with PTC is unclear. These findings support further investigation of the prognostic and therapeutic implications of BRAF V600E status in PTC.

Full text available from *Journal of the American Medical Association* (USD 30.00)
PMID: 23571588

3.12 Other Tumors

3.12.1 Soft Tissue

A) Molecular Diagnostics of Soft Tissue Tumors
Bridge JA, Cushman-Vokoun AM. Molecular diagnostics of soft tissue tumors. *Arch Pathol Lab Med*. 2011 May; 135(5):588–601.

Summary: Soft tissue pathology encompasses a remarkably diverse assortment of benign and malignant soft tissue tumors. Rendering a definitive diagnosis is complicated not only by the large volume of existing histologic subtypes (>100) but also frequently by the presence of overlapping clinical, histologic, immunohistochemical, and/or radiographic features. During the past 3 decades,

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mesenchymal tumor–specific, cytogenetic and molecular genetic abnormalities have demonstrated an increasingly important, ancillary role in mesenchymal tumor diagnostics. Objectives.—To review molecular diagnostic tools available to the pathologist to further classify specific soft tissue tumor types and recurrent aberrations frequently examined. Advantages and limitations of individual approaches will also be highlighted. Data Sources.—Previously published review articles, peer-reviewed research publications, and the extensive cytogenetic and molecular diagnostic experience of the authors to include case files of The University of Nebraska Medical Center. Conclusions.—Cytogenetic and molecular genetic assays are used routinely for diagnostic purposes in soft tissue pathology and represent a powerful adjunct to complement conventional microscopy and clinicoradiographic evaluation in the formulation of an accurate diagnosis. Care should be taken, however, to recognize the limitations of these approaches. Ideally, more than one technical approach should be available to a diagnostic laboratory to compensate for the shortcomings of each approach in the assessment of individual specimens.

Free full text available from CAP's *Archives*
PMID: 21526957
NOTE: Also cited in Section 8.4

3.12.2 Squamous and Salivary Gland

A) An Update on Molecular Diagnostics of Squamous and Salivary Gland Tumors of the Head and Neck

Hunt JL. An update on molecular diagnostics of squamous and salivary gland tumors of the head and neck. *Arch Pathol Lab Med*. 2011 May; 135(5):602–609.

Summary: Molecular testing in anatomic pathology is becoming standardized and can contribute valuable diagnostic, therapeutic, and prognostic information for the clinical management of patients. In head and neck pathology, recent advances in molecular testing have provided important targets in several different diagnostic areas, with particular emerging clinical applications in squamous and salivary

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gland pathology. In squamous mucosal-derived lesions, human papilloma virus has emerged as an important pathogenic etiology in a subset of oropharyngeal squamous cell carcinomas. Within the category of salivary gland tumors, 3 tumors have recently been recognized that contain oncogenic translocations. Objective.—To describe the current state of information about the molecular alterations in squamous lesions and in salivary gland tumors of the head and neck. Data Sources.—Published literature on squamous and salivary gland tumors of the head and neck. Conclusions.—The different approaches to identification of viral-associated tumors include assays using polymerase chain reaction, in situ hybridization, and immunohistochemistry. Most mucoepidermoid carcinomas harbor MECT1-MAML2 gene rearrangement. The MYB-NFIB translocations have recently been identified in adenoid cystic carcinomas. Finally, a newly described tumor of salivary gland, mammary analogue secretory carcinoma, harbors the ETV6-NTRK3 translocation. Although these translocations are just emerging as diagnostic targets, future roles may evolve as potential therapeutic targets.

Free full text available from the CAP's *Archives*
PMID: 21526958

3.12.3 Solid Tumors – Other Considerations

A) Molecular Testing of Solid Tumors
Igbokwe A, Lopez-Terrada DH. Molecular testing of solid tumors. *Arch Pathol Lab Med.* 2011 January; 135(1):67–82.

Summary: Molecular testing of solid tumors is steadily becoming a vital component of the contemporary anatomic pathologist's armamentarium. These sensitive and specific ancillary tools are useful for confirming ambiguous diagnoses suspected by light microscopy and for guiding therapeutic decisions, assessing prognosis, and monitoring patients for residual neoplastic disease after therapy. Objective—To review current molecular biomarkers and tumor-specific assays most useful in solid tumor testing, specifically of breast, colon, lung, thyroid, and soft tissue tumors, malignant melanoma, and tumors of unknown origin. A few upcoming molecular

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diagnostic assays that may become standard of care in the near future will also be discussed. Data Sources—Original research articles, review articles, and the authors' personal practice experience. Conclusions—Molecular testing in anatomic pathology is firmly established and will continue to gain ground as the need for more specific diagnoses and new targeted therapies evolve. Knowledge of the more common and clinically relevant molecular tests available for solid tumor diagnosis and management, and their indications and limitations, is necessary if anatomic pathologists are to optimally use these tests and act as consultants for fellow clinicians directly involved in patient care.

Free full text available from the CAP's *Archives*
PMID: 21204713
NOTE: *Also cited in Section 3.1*

B) Circulating Tumor Cells (CTCs)

Technology Assessment Committee. College of American Pathologists. Circulating tumor cells (CTCs). December 17, 2010. http://www.cap.org/apps/cap.portal?_nfpb=true&cntvwrPtlt_actionOverride=%2Fportlets%2FcontentViewer%2Fshow&_windowLabel=cntvwrPtlt&cntvwrPtlt%7BactionForm.contentReference%7D=committees%2Ftechnology%2Fctc.html&_state=maximized&_pageLabel=cntvwr. Accessed January 19, 2012.

Summary: The ability to detect circulating tumor cells (CTCs) in a whole blood specimen has the potential to significantly influence the practice of pathology. Its application includes risk (prognosis) stratification of cancer patients, early detection of relapse, response monitoring to treatment in patients with metastatic carcinoma, and if the CTCs can be acquired for phenotypic and/or genotypic analysis, they can potentially improve therapy selection and develop novel targeted therapies. Developed by the Technology Assessment Committee (TAC), Perspectives on Emerging Technology (POET) reports and white papers are designed to provide pathologists with a high-level summary of a particular emerging technology that is likely to impact their practice in the reasonable future. POET reports help pathologists respond to clinician or patient inquiries about a technology. Its format includes a one-page summary plus select

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references (e.g., peer-reviewed articles, for further information and research.) Although POETs deliver a short overview of a specific innovative technology, they are not a definitive technology assessment of the techniques used or a “how to” cookbook on implementing a test in a practice. Rather, they are intended to be used as an educational tool leading to a more detailed investigation by the Center, Council on Scientific Affairs, TAC or individual pathologists.

CTCs POET Report; POET Reports homepage

C) Analysis of Circulating Tumor DNA to Monitor Metastatic Breast Cancer

Dawson SJ, Tsui DW, Murtaza M, et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. *N Engl J Med.* 2013 Mar 28; 368(13): 1199-1209.

Summary: The management of metastatic breast cancer requires monitoring of the tumor burden to determine the response to treatment, and improved biomarkers are needed. Biomarkers such as cancer antigen 15-3 (CA 15-3) and circulating tumor cells have been widely studied. However, circulating cell-free DNA carrying tumor-specific alterations (circulating tumor DNA) has not been extensively investigated or compared with other circulating biomarkers in breast cancer. METHODS: We compared the radiographic imaging of tumors with the assay of circulating tumor DNA, CA 15-3, and circulating tumor cells in 30 women with metastatic breast cancer who were receiving systemic therapy. We used targeted or whole-genome sequencing to identify somatic genomic alterations and designed personalized assays to quantify circulating tumor DNA in serially collected plasma specimens. CA 15-3 levels and numbers of circulating tumor cells were measured at identical time points. RESULTS: Circulating tumor DNA was successfully detected in 29 of the 30 women (97%) in whom somatic genomic alterations were identified; CA 15-3 and circulating tumor cells were detected in 21 of 27 women (78%) and 26 of 30 women (87%), respectively. Circulating tumor DNA levels showed a greater dynamic range, and greater correlation with changes in tumor burden, than did CA 15-3 or circulating tumor cells. Among the measures tested, circulating tumor

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DNA provided the earliest measure of treatment response in 10 of 19 women (53%). CONCLUSIONS: This proof-of-concept analysis showed that circulating tumor DNA is an informative, inherently specific, and highly sensitive biomarker of metastatic breast cancer. (Funded by Cancer Research UK and others.).

Full text available from *New England Journal of Medicine* (USD 15.00)
PMID: 23484797

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Section 4 Molecular Diagnostics for Hereditary Diseases

4.1 Choosing Which Molecular Tests to Perform/Interpret

A) Test Verification and Validation for Molecular Diagnostic Assays

Halling KC, Schrijver I, Persons DL. Test verification and validation for molecular diagnostic assays. *Arch Pathol Lab Med.* 2012 Jan;136(1):11-3.

Summary: With our ever-increasing understanding of the molecular basis of disease, clinical laboratories are implementing a variety of molecular diagnostic tests to aid in the diagnosis of hereditary disorders, detection and monitoring of cancer, determination of prognosis and guidance for cancer therapy, and detection and monitoring of infectious diseases. Before introducing any new test into the clinical laboratory, the performance characteristics of the assay must be verified," if it is a US Food and Drug Administration (FDA)-approved or FDA-cleared test, or "validated," if it is a laboratory-developed test. Although guidelines exist for how validation and verification studies may be addressed for molecular assays, the specific details of the approach used by individual laboratories is rarely published. Many laboratories, especially those introducing new types of molecular assays, would welcome additional guidance, especially in the form of specific examples, on the process of preparing a new molecular assay for clinical use.

Free full text available from the CAP's *Archives*
PMID: 22208481

B) Chromosomal Microarray Testing Influences Medical Management

Coulter ME, Miller DT, Harris DJ, et al. Chromosomal microarray testing influences medical management. *Genet Med*. 2011 Sep;13(9):770-6.

Summary: Chromosomal microarray (CMA) testing provides the highest diagnostic yield for clinical testing of patients with developmental delay (DD), intellectual disability (ID), multiple congenital anomalies (MCA), and autism spectrum disorders (ASD). Despite improved diagnostic yield and studies to support cost-effectiveness, concerns regarding the cost and reimbursement for CMA have been raised because it is perceived that CMA results do not influence medical management. METHODS: We conducted a retrospective chart review of CMA testing performed during a 12-month period on patients with DD/ID, ASD, and congenital anomalies to determine the proportion of cases where abnormal CMA results impacted recommendations for clinical action. RESULTS: Among 1792 patients, 13.1% had clinically relevant results, either abnormal (n = 131; 7.3%) or variants of possible significance (VPS; n = 104; 5.8%). Abnormal variants generated a higher rate of recommendation for clinical action (54%) compared with VPS (34%; Fisher exact test, P = 0.01). CMA results influenced medical care by precipitating medical referrals, diagnostic imaging, or specific laboratory testing. CONCLUSIONS: For all test indications, CMA results influenced medical management in a majority of patients with abnormal variants and a substantial proportion of those with VPS. These results support the use of CMA as a clinical diagnostic test that influences medical management for this patient population.

Full text available for purchase from *Genetics in Medicine* (USD 32.00)
PMID: 21716121

C) Good Laboratory Practices for Molecular Genetic Testing for Heritable Diseases and Conditions

Chen B, Gagnon M, Shahangian S, Anderson NL, Howerton DA, Boone JD; Centers for Disease Control and Prevention (CDC). Good laboratory practices for molecular genetic testing for heritable

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diseases and conditions. *MMWR Recomm Rep*. 2009 Jun 12;58(RR-6):1-37; quiz CE-1-4.

Summary: Under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) regulations, laboratory testing is categorized as waived (from routine regulatory oversight)or nonwaived based on the complexity of the tests; tests of moderate and high complexity are nonwaived tests. Laboratories that perform molecular genetic testing are subject to the general CLIA quality systems requirements for nonwaived testing and the CLIA personnel requirements for tests of high complexity. Although many laboratories that perform molecular genetic testing comply with applicable regulatory requirements and adhere to professional practice guidelines,specific guidelines for quality assurance are needed to ensure the quality of test performance. To enhance the oversight of genetic testing under the CLIA framework,CDC and the Centers for Medicare & Medicaid Services (CMS) have taken practical steps to address the quality management concerns in molecular genetic testing,including working with the Clinical Laboratory Improvement Advisory Committee (CLIAC). This report provides CLIAC recommendations for good laboratory practices for ensuring the quality of molecular genetic testing for heritable diseases and conditions. The recommended practices address the total testing process (including the preanalytic,analytic,and postanalytic phases),laboratory responsibilities regarding authorized persons,confidentiality of patient information,personnel competency,considerations before introducing molecular genetic testing or offering new molecular genetic tests,and the quality management system approach to molecular genetic testing. These recommendations are intended for laboratories that perform molecular genetic testing for heritable diseases and conditions and for medical and public health professionals who evaluate laboratory practices and policies to improve the quality of molecular genetic laboratory services. This report also is intended to be a resource for users of laboratory services to aid in their use of molecular genetic tests and test results in health assessment and care. Improvements in the quality and use of genetic laboratory services should improve the quality of health care and health outcomes for patients and families of patients.

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Free full text available from Centers for Disease Control and Prevention
PMID: 19521335

4.2 Patient Care

4.2.1 Quick Reference Table: Commonly Tested Genes for Hereditary Disease

Hereditary Disease	Gene
Thrombophilia	F2 (Prothrombin), F5 (Factor V), others
Cystic Fibrosis	CFTR
Familial Adenomatous Polyposis	APC, MUTYH
Hereditary breast and ovarian CA	BRCA1, BRCA2, PTEN, TP53, STK11, CDH1, others
Intellectual Disability (ID) testing	
• Fragile X syndrome	FMR1 repeat expansion
• Rett Syndrome	MECP2
• PTEN-Related Disorders	PTEN
• Angelman and Prader-Willi Syndromes	Deletion/abnormal methylation of Chr 15, others
• Noonan Syndrome	PTPN11, SOS1, RAF1, KRAS, CBL, RIT1
Beckwith-Wiedemann Syndrome	Abnormal Methylation Chr 11p, others
Achondroplasia	FGFR3
Hypochondroplasia	FGFR3
Thanatophoric Dysplasia	FGFR3
Hemochromatosis	HFE, others
Hemoglobinopathies	Alpha Globin (HBA1, HBA2), Beta Globin (HBB)
Hemolytic Anemia due to G6PD deficiency	G6PD
Hemophilia A/B	F8 (Factor VIII), F9 (Factor IX)
Hereditary Pancreatitis	PRSS1, SPINK1, CFTR

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Huntington Disease	HTT repeat expansion
Inborn Errors of Metabolism	
• Lysosomal storage diseases	GBA, HEXA, SMPD1, GLA, others
• Fatty acid oxidation disorders	ACADM (MCAD), ACADVL (VLCAD)
• Galactosemia	GALT
• Biotinidase deficiency	BTD
• PKU	PAH
• Urea Cycle and related disorders	OTC, ASS1, CPS1, ASL, ARG1, NAGS, SLC25A13
Juvenile Polyposis	BMPR1A, SMAD4
Lynch Syndrome and Constitutional mismatch repair deficiency	MLH1, MSH2, MSH6, PMS2
Marfan Syndrome	FBN1
Muscular Dystrophy, Duchenne type	DMD
Multiple Endocrine Neoplasia Type 1, Type 2	MEN1, RET
Myotonic Dystrophy	DMPK repeat expansion
Neurofibromatisis Type 1	NF1
Nonsyndromic hearing loss	GJB2 (Connexin 26), GJB6, many others
Pheochromocytoma	SDHB, SDHC, SDHD, VHL, RET, MAX, TMEM127, others
Polycystic kidney disease	PKD1, PKD2, others
Rh Genotyping (maternal alloimmunization)	RHD, RHCE
Renal Cell Carcinoma	VHL, MET, FLCN, FH, others
Retinoblastoma	RB1
Spinal Muscular Atrophy	SMN1, SMN2 (copy number)
Tuberous Sclerosis	TSC1, TSC2
von Willebrand Disease	VWF
Wilms Tumor	WT1, Abnormal Methylation Chr 11p, others

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4.2.2 Genetic Counseling

A) The Coming Explosion in Genetic Testing--Is There a Duty to Recontact?

Pyeritz RE. The coming explosion in genetic testing--is there a duty to recontact? *N Engl J Med.* 2011 Oct 13; 365(15):1367–9.

Summary: The question of whether a duty exists to recontact patients about new genetic information has been debated for several decades without consensus, but the emergence of new technologies compels us to reconsider this complex matter. Ordering a “genetic test,” such as a chromosome analysis or a search for a mutation, is different from ordering a complete blood count. Before obtaining a specimen, counseling of the patient is required in order to discuss confidentiality, potential anxiety, stigma or discrimination, the interpretation and implications of possible results, and relevant follow-up options. Ideally, both pre- and post-test counseling would be conducted by genetic counselors, but there aren't enough such professionals to meet the current demands. Uncertainty in the results of many genetic tests, such as gene sequencing and cytogenomic arrays, presents a conundrum. A result may be abnormal and clearly pathologic, reflecting a disease that is present, a disease that will appear later, or a susceptibility to a common disease. Conversely, depending on the thoroughness of the analysis, a “negative” result may not mean that the patient doesn't have a mutation. Perhaps the most confounding outcomes are “variants of unknown significance” — the primary findings in 5 to 30% of all gene-sequencing results. The complexities inherent in genetic testing will expand markedly as whole-genome sequencing becomes more widespread.1 The thousands of whole-genome analyses performed to date have all revealed clear mutations in several genes and many variants of unknown significance.2 The clinical implications of much genetic variation will eventually become clearer, but today uncertainty is the rule. Emphasizing this point, and the need for rigorous pre- and post-test counseling, does not, in my view, constitute genetic exceptionalism. These are facts of life. Determining whether, when, and how to recontact patients when the interpretation of their genetic test changes involves ethical, legal, and practical considerations. Ten salient issues should stimulate and inform the debate. Most require further investigation.

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Full text available from *New England Journal of Medicine* (USD 15.00)
PMID: 21995382

B) Value of Genetic Counselors in the Laboratory

Miller CE, Kraustcheid P, Baldwin EE, LaGrave D, Openshaw A, Hart K, Tvrdik T; ARUP Laboratories. Value of Genetic Counselors in the Laboratory. March, 2011. http://www.aruplab.com/files/resources/genetics/White-paper-1-value-of-GCs-in-lab.pdf. Accessed September 26, 2013.

Summary: Genetic counselors (GCs) employed by diagnostic laboratories may write medical papers, coordinate research, create and maintain genetic databases, educate clients and health care providers, and review test orders. Of these duties, the one that most directly benefits patients, medical institutions, and insurers is the rigorous reviewing of genetic test orders. GCs at ARUP Laboratories, a national reference laboratory, collectively save ordering institutions more than $30,000 per month by modifying test orders to improve utilization. Seven GCs at ARUP Laboratories performed a review of all genetic test modifications over an 11-month period, reviewing clinical information that accompanied test orders for complex genetic tests (i.e., sequencing, large duplication/deletion analysis, or arraybased technologies) before testing was performed. The GCs considered the clinical utility and cost-effectiveness of the ordered tests and contacted the ordering institution and/or health care provider to collect additional clinical information, confirm testing, or suggest alternative testing based on the provided clinical information or family history. The GCs identified and cancelled or changed inappropriately ordered genetic tests for an average cost savings of $36,500 per month, representing approximately 30 percent of all complex genetic tests ordered. Among frequently misordered tests were requests for fullgene sequencing when a familial mutation was known or when a screening panel would have been more appropriate (e.g., cystic fibrosis testing in expectant individuals with no family history). Erroneously ordered genetic testing delays medical decision-making and increases diagnostic costs. In 2008, U.S. health care spending was the highest of all industrialized countries, about $7,681 per resident, and accounted for 16.2 percent of the nation's gross

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domestic product (GDP). Reducing the growth in health care costs is thus a priority.

Free full text available from ARUP Laboratories

4.3 Hereditary Diseases

A) Gene Reviews

Pagon RA, Bird TD, Dolan CR, Stephens K, Adam MP, editors. *GeneReviews*™ [Internet]. Seattle, WA: University of Washington, Seattle; 1993.

Summary: GeneReviews are expert-authored, peer-reviewed disease descriptions that apply genetic testing to the diagnosis, management, and genetic counseling of patients and families with specific inherited conditions. Published exclusively online, each GeneReview entry is: (1) peer reviewed for accuracy by (a) editorial staff experts in clinical genetics, laboratory genetics, and genetic counseling and by (b) acknowledged international subject experts; (2) updated by the author(s) in a formal comprehensive process every two to three years or as needed; and (3) revised by the author(s) or editorial staff whenever significant changes in clinically relevant information occur. GeneReviews are part of the GeneTests Web site, which also includes: a Laboratory Directory of US and international laboratories offering molecular genetic testing, specialized cytogenetic testing, and biochemical testing for inherited disorders; a Clinic Directory of US and international genetics clinics providing genetic evaluation and genetic counseling; Resources that are consumer health-oriented organizations and disease registries; Educational materials; and an Illustrated Glossary of genetic counseling and testing terms.

Free full article available from *GeneReviews*

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B) Development of Genomic Reference Materials for Cystic Fibrosis Genetic Testing

Pratt VM, Caggana M, Bridges C, et al. Development of genomic reference materials for cystic fibrosis genetic testing. *J Mol Diagn*. 2009 May;11(3):186-93.

Summary: The number of different laboratories that perform genetic testing for cystic fibrosis is increasing. However, there are a limited number of quality control and other reference materials available, none of which cover all of the alleles included in commercially available reagents or platforms. The alleles in many publicly available cell lines that could serve as reference materials have neither been confirmed nor characterized. The Centers for Disease Control and Prevention-based Genetic Testing Reference Material Coordination Program, in collaboration with members of the genetic testing community as well as Coriell Cell Repositories, have characterized an extended panel of publicly available genomic DNA samples that could serve as reference materials for cystic fibrosis testing. Six cell lines [containing the following mutations: E60X (c.178G>T), 444delA (c.312delA), G178R (c.532G>C), 1812-1G>A (c.1680-1G>A), P574H (c.1721C>A), Y1092X (c.3277C>A), and M1101K (c.3302T>A)] were selected from those existing at Coriell, and seven [containing the following mutations: R75X (c.223C>T), R347H (c.1040G>A), 3876delA (c.3744delA), S549R (c.1646A>C), S549N (c.1647G>A), 3905insT (c.3773_3774insT), and I507V (c.1519A>G)] were created. The alleles in these materials were confirmed by testing in six different volunteer laboratories. These genomic DNA reference materials will be useful for quality assurance, proficiency testing, test development, and research and should help to assure the accuracy of cystic fibrosis genetic testing in the future. The reference materials described in this study are all currently available from Coriell Cell Repositories.

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PMID: 19359498

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C) Cystic Fibrosis Population Carrier Screening: 2004 Revision of American College of Medical Genetics Mutation Panel

Watson MS, Cutting GR, Desnick RJ, et al. Cystic fibrosis population carrier screening: 2004 revision of American College of Medical Genetics mutation panel. *Genet Med.* 2004 Sep-Oct; 6(5): 387-391.

Summary: In April 2001, the American College of Medical Genetics (ACMG) Cystic Fibrosis (CF) Carrier Screening Working Group recommended a panel of mutations and variants that should be tested to determine carrier status within the *CFTR* gene as a part of population screening programs.[1,2] This was initially done in response to the recommendations of an NIH CF Consensus Conference that CF carrier screening be considered by all couples for use before conception or prenatally.[3] At that time, the Working Group recognized limitations in our understanding of the population frequencies of several CF alleles and proposed to review mutation distribution data after the first two years of the program. In 2002, as part of an ongoing effort to ensure that the cystic fibrosis carrier screening programs are current with respect to the scientific literature and other available data and practices, we initiated a second review of data on the distribution of mutations in different ethnic groups and we began to assess whether providers were experiencing challenges in delivering this service.[4] The current CF Foundation patient mutation database includes nearly double the number of CF patient chromosomes available for analysis in 2000. This report summarizes the major recommendations of our Working Group with the supporting justification for these decisions. A number of articles in this issue of *Genetics in Medicine* provide some of the data on which our decisions were made, whereas others provide new information related to this topic.

Free full text available from PubMed
PMID: 15371902

D) Good Laboratory Practices for Biochemical Genetic Testing and Newborn Screening for Inherited Metabolic Disorders

Centers for Disease Control and Prevention. Good laboratory practices for biochemical genetic testing and newborn screening for

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inherited metabolic disorders. *MMWR Recomm Rep.* 2012 Apr 6; 61(RR-2): 1-44.

Summary: Biochemical genetic testing and newborn screening are essential laboratory services for the screening, detection, diagnosis, and monitoring of inborn errors of metabolism or inherited metabolic disorders. Under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) regulations, laboratory testing is categorized on the basis of the level of testing complexity as either waived (i.e., from routine regulatory oversight) or nonwaived testing (which includes tests of moderate and high complexity). Laboratories that perform biochemical genetic testing are required by CLIA regulations to meet the general quality systems requirements for nonwaived testing and the personnel requirements for high-complexity testing. Laboratories that perform public health newborn screening are subject to the same CLIA regulations and applicable state requirements. As the number of inherited metabolic diseases that are included in state-based newborn screening programs continues to increase, ensuring the quality of performance and delivery of testing services remains a continuous challenge not only for public health laboratories and other newborn screening facilities but also for biochemical genetic testing laboratories. To help ensure the quality of laboratory testing, CDC collaborated with the Centers for Medicare & Medicaid Services, the Food and Drug Administration, the Health Resources and Services Administration, and the National Institutes of Health to develop guidelines for laboratories to meet CLIA requirements and apply additional quality assurance measures for these areas of genetic testing. This report provides recommendations for good laboratory practices that were developed based on recommendations from the Clinical Laboratory Improvement Advisory Committee, with additional input from the Secretary's Advisory Committee on Genetics, Health, and Society; the Secretary's Advisory Committee on Heritable Disorders in Newborns and Children; and representatives of newborn screening laboratories. The recommended practices address the benefits of using a quality management system approach, factors to consider before introducing new tests, establishment and verification of test performance specifications, the total laboratory testing process (which consists of the preanalytic, analytic, and postanalytic phases), confidentiality of patient information and test results, and personnel

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qualifications and responsibilities for laboratory testing for inherited metabolic diseases. These recommendations are intended for laboratories that perform biochemical genetic testing to improve the quality of laboratory services and for newborn screening laboratories to ensure the quality of laboratory practices for inherited metabolic disorders. These recommendations also are intended as a resource for medical and public health professionals who evaluate laboratory practices, for users of laboratory services to facilitate their collaboration with newborn screening systems and use of biochemical genetic tests, and for standard-setting organizations and professional societies in developing future laboratory quality standards and practice recommendations. This report complements Good Laboratory Practices for Molecular Genetic Testing for Heritable Diseases and Conditions (CDC. Good laboratory practices for molecular genetic testing for heritable diseases and conditions. MMWR 2009;58 [No. RR-6]) to provide guidance for ensuring and improving the quality of genetic laboratory services and public health outcomes. Future recommendations for additional areas of genetic testing will be considered on the basis of continued monitoring and evaluation of laboratory practices, technology advancements, and the development of laboratory standards and guidelines.

Free full text available from *Morbidity and Mortality Weekly Report*
PMID: 22475884

E) Clinical Guidelines for Testing for Heritable Thrombophilia
Baglin T, Gray E, Greaves M, et al. Clinical guidelines for testing for heritable thrombophilia. *Br J Haematol.* 2010 Apr; 149(2): 209-220.

Summary: The guideline group was selected to be representative of UK-based medical experts. The writing group met and communicated by email. The guideline was reviewed by a multidisciplinary sounding board, selected non-UK experts in thrombosis and thrombophilia, the British Committee for Standards in Haematology (BCSH) and the British Society for Haematology) (BSH and comments incorporated where appropriate. Criteria used to quote levels and grades of evidence are according to the GRADE system (Guyatt et al, 2006). As this guideline relates specifically to laboratory tests, reference is made to grading quality of evidence and strength of recommendations for

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diagnostic tests and strategies recognising that tests are only of value if they result in improved outcomes for patients (Schunemann et al, 2008). Strong recommendations (grade 1, 'recommended') are made when there is confidence that the benefits either do or do not outweigh the harm and burden and costs of treatment. Where the magnitude of benefit or not is less certain, a weaker grade 2 recommendation ('suggested') is made. Grade 1 recommendations can be applied uniformly to most patients whereas grade 2 recommendations require judicious application. The quality of evidence is graded as A (high quality randomised clinical trials), moderate (B) or low (C) (Guyatt et al, 2006; http://www.bcshguidelines.com). The target audience for this guideline is healthcare professionals involved in the management of patients and families with venous thrombosis or pregnancy morbidity.

Free full text available from *British Journal of Haematology*
PMID: 20128794

F) ACMG Practice Guideline: Lack of Evidence for MTHFR Polymorphism Testing

Hickey SE, Curry CJ, Toriello HV. ACMG Practice Guideline: lack of evidence for MTHFR polymorphism testing. *Genet Med.* 2013 Feb; 15(2): 153-156.

Summary: MTHFR polymorphism testing is frequently ordered by physicians as part of the clinical evaluation for thrombophilia. It was previously hypothesized that reduced enzyme activity of MTHFR led to mild hyperhomocysteinemia which led to an increased risk for venous thromboembolism, coronary heart disease, and recurrent pregnancy loss. Recent meta-analyses have disproven an association between hyperhomocysteinemia and risk for coronary heart disease and between MTHFR polymorphism status and risk for venous t-hromboembolism. There is growing evidence that MTHFR polymorphism testing has minimal clinical utility and, therefore should not be ordered as a part of a routine evaluation for thrombophilia.

Full text available from *Genetics in Medicine* (USD 32.00)
PMID: 23288205

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G) Current Molecular Diagnostic Algorithm for Mitochondrial Disorders

Wong LJ, Scaglia F, Graham BH, Craigen WJ. Current molecular diagnostic algorithm for mitochondrial disorders. *Mol Genet Metab.* 2010 Jun; 100(2): 111-117.

Summary: Mitochondrial respiratory chain disorders (RCD) are a group of genetically and clinically heterogeneous diseases, due in part to the biochemical complexity of mitochondrial respiration and the fact that two genomes, one mitochondrial and one nuclear, encode the components of the respiratory chain. Because of the large number of genes involved, attempts to classify mitochondrial RCD incorporate clinical, biochemical, and histological criteria, in addition to DNA-based molecular diagnostic testing. While molecular testing is widely viewed as definitive, confirmation of the diagnosis by molecular methods often remains a challenge because of the large number of genes, the two genome complexity and the varying proportions of pathogenic mitochondrial DNA (mtDNA) molecules in a patient, a concept termed heteroplasmy. The selection of genes to be analyzed depends on the family history and clinical, biochemical, histopathological, and imaging results, as well as the availability of different tissues for analysis. Screening of common point mutations and large deletions in mtDNA is typically the first step. In cases where tissue-specific, recognizable clinical syndromes or characteristic RC complex deficiencies and histochemical abnormalities are observed, direct sequencing of the specific causative nuclear gene(s) can be performed on white blood cell DNA. Measurement of mtDNA content in affected tissues such as muscle and liver allows screening for mtDNA depletion syndromes. The ever-expanding list of known disease-causing genes will undoubtedly improve diagnostic accuracy and genetic counseling.

Full text available from *Molecular Genetics and Metabolism* (USD 41.95)
PMID: 20359921

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4.4 Epigenetics

A) DNA Methylation Testing and Marker Validation Using PCR: Diagnostic Applications

Egger G, Wielscher M, Pulverer W, Kriegner A, Weinhäusel A. DNA methylation testing and marker validation using PCR: diagnostic applications. *Expert Rev Mol Diagn.* 2012 Jan; 12(1): 75-92.

Summary: DNA methylation provides a fundamental epigenetic mechanism to establish and promote cell-specific gene-expression patterns, which are inherited by subsequent cell generations. Thus, the epigenome determines the differentiation into a cell lineage but can also program cells to become abnormal or malignant. In humans, different germline and somatic diseases have been linked to faulty DNA methylation. In this article, we will discuss the available PCR-based technologies to assess differences in DNA methylation levels mainly affecting 5-methylcytosine in the CpG dinucleotide context in hereditary syndromal and somatic pathological conditions. We will discuss some of the current diagnostic applications and provide an outlook on how DNA methylation-based biomarkers might provide novel tools for diagnosis, prognosis or patient stratification for diseases such as cancer.

Full text available from *Expert Review of Molecular Diagnostics* (USD 60.00)
PMID: 22133121

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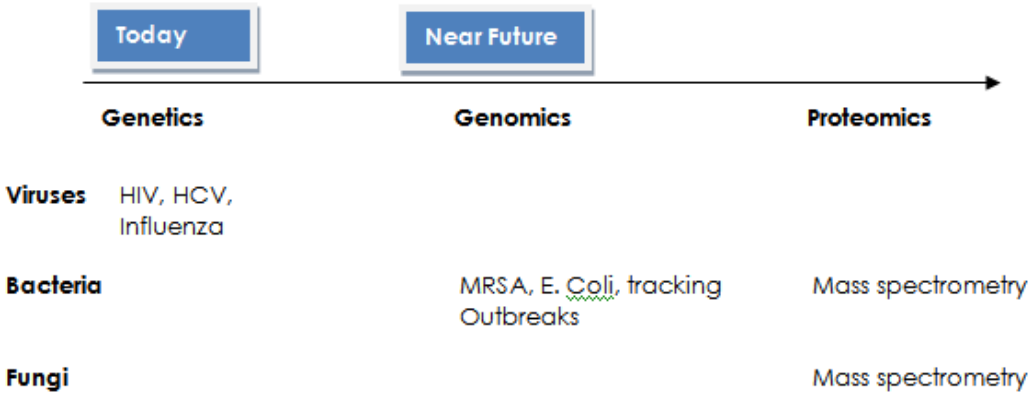
Section 5 Molecular Diagnostics for Infectious Diseases

5.1 Overview



Molecular diagnostic techniques have been used to identify the presence of infectious agents in humans for over 20 years. In 1996, the Patient Preparation and Specimen Handling Editorial Board of the College of American Pathologists published fascicle VII entitled Reference Guide for Diagnostic Pathology / Flow Cytometry**.** Of the 134 entries, 50 defined the detection of infectious diseases using molecular testing including 18 viruses, 18 bacteria, 12 parasites and 2 fungi. The field has made great progress since then with the introduction of additional assays using biplex (herpes simplex viruses I and II) or multiplex assays like respiratory virus panels or gastric pathogen panels. The introduction of those assays which require a rapid turnaround time in the local hospital locale provides improved patient care and outcomes and associated reduced length of stay and cost avoidance. Therefore, this group of assays is often considered as the place to start when initially planning a molecular diagnostics laboratory. This brief introduction to 7 viral and 4 bacterial assays provides a foundation onto which additional assays may be introduced to your molecular laboratories test list.

Section 5

A) Nucleic Acid Based Tests: List of Microbial Tests

U. S. Food and Drug Administration. Nucleic Acid Based Tests: List of Microbial Tests. Food and Drug Administration Web site. http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm#microbial. Accessed June 28, 2013.

Summary: Nucleic acid-based testing for infectious diseases now encompasses a broad menu of FDA-cleared in vitro diagnostic assays. A current list is maintained on the website of the FDA, for which the link is included below. A variety of testing indications are represented including diagnosis of sexually transmitted diseases, respiratory viruses, Clostridium difficile, MRSA, mycobacterial species, and quantification of HCV, HBV, and HIV, among others.

Access FDA Table

B) Diagnosing Emerging and Reemerging Infectious Diseases: The Pivotal Role of the Pathologist

Olano JP, Walker DH. Diagnosing emerging and reemerging infectious diseases: the pivotal role of the pathologist. *Arch Pathol Lab Med*. 2011 Jan; 135(1):83–91.

Summary: Molecular diagnostics continues to evolve very rapidly, and its impact in the diagnosis of infectious diseases is undeniable. Molecular tools have played a pivotal role in discovering and characterizing several emerging infectious agents and have now become the gold standard for the diagnosis of infectious diseases caused by fastidious or uncultivable agents. Multiple challenges still remain for the widespread use of cost-effective, validated, and commercially available molecular tools. Automated instruments capable of sample processing and multiplex nucleic acid amplification and postamplification analysis have already been approved by the US Food and Drug Administration (FDA) for use in the clinical setting. Nanobiotechnology is beginning to impact laboratory diagnostics in the clinical setting. Objective—To address current nucleic acid techniques used in the clinical laboratory for diagnosis of infectious diseases. FDA-approved tests are listed, as well as molecular techniques (amplification and postamplification analysis). A comprehensive list of emerging pathogens during the last 4 decades

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is also presented. Biosurveillance systems are discussed in the context of molecular tools. The rapidly evolving field of nanobiotechnology is briefly addressed. Data Sources—Original publications, major reviews, and book chapters were used to present a comprehensive, yet short, review of molecular diagnostics in infectious diseases. Conclusions—We will continue to witness an exponential growth of molecular techniques used for the initial diagnosis of infectious diseases. Molecular tools will also continue to have an impact on disease prognosis and response to therapeutic interventions. Automation, multiplexing, and miniaturization will continue to be driving forces in the development of new instruments.

Free full text available from the CAP's *Archives*
PMID: 21204714

C) Structure, Function and Diversity of the Healthy Human Microbiome

Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012 Jun 13;486(7402):207-14. doi:10.1038/nature11234.

Summary: Studies of the human microbiome have revealed that even healthy individuals differ remarkably in the microbes that occupy habitats such as the gut, skin and vagina. Much of this diversity remains unexplained, although diet, environment, host genetics and early microbial exposure have all been implicated. Accordingly, to characterize the ecology of human-associated microbial communities, the Human Microbiome Project has analysed the largest cohort and set of distinct, clinically relevant body habitats so far. We found the diversity and abundance of each habitat's signature microbes to vary widely even among healthy subjects, with strong niche specialization both within and among individuals. The project encountered an estimated 81-99% of the genera, enzyme families and community configurations occupied by the healthy Western microbiome. Metagenomic carriage of metabolic pathways was stable among individuals despite variation in community structure, and ethnic/racial background proved to be one of the strongest associations of both pathways and microbes with clinical metadata. These results thus delineate the range of structural and functional configurations normal

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in the microbial communities of a healthy population, enabling future characterization of the epidemiology, ecology and translational applications of the human microbiome.

Free full text available from *Nature*
PMID: 22699609

5.1.1 Setting up an Infectious Disease Molecular Testing Lab

A) International Standards and Reference Materials for Quantitative Molecular Infectious Disease Testing

Madej RM, Davis J, Holden MJ, Kwang S, Labourier E, Schneider GJ. International standards and reference materials for quantitative molecular infectious disease testing. *J Mol Diagn*. 2010 Mar;12(2):133-43. Epub 2010 Jan 14.

Summary: The utility of quantitative molecular diagnostics for patient management depends on the ability to relate patient results to prior results or to absolute values in clinical practice guidelines. To do this, those results need to be comparable across time and methods, either by producing the same value across methods and test versions or by using reliable and stable conversions. Universally available standards and reference materials specific to quantitative molecular technologies are critical to this process but are few in number. This review describes recent history in the establishment of international standards for nucleic acid test development, organizations involved in current efforts, and future issues and initiatives.

Free full text available from PubMed
PMID: 20075208

B) Molecular Detection and Surveillance of Healthcare Associated Infections

Rao A, Fader B, Hocker K. Molecular detection and surveillance of healthcare association infections. In: Grody WW, Nakamura RM, Kiechle FL, eds. *Molecular Diagnostics: Techniques and Applications*

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for the Clinical Laboratory. Boston, MA: Academic Press; 2010: 327-346.

Summary: Describes health care related infections that are commonly associated with stays in hospitals or other healthcare facilities. These may be urinary tract infections, bloodstream infections, surgical site infections and pneumonia. Associated organisms include *Staphylococcus aureus, Enterococci, Clostridium difficile,* and *Candida* as well as others. The chapter also discusses the costs of caring for infected patients.

Book available for purchase from Google

C) Validation of Laboratory-Developed Molecular Assays for Infectious Diseases

Burd EM. Validation of laboratory-developed molecular assays for infectious diseases. *Clin Microbiol Rev.* 2010 Jul; 23(3): 550-576.

Summary: Molecular technology has changed the way that clinical laboratories diagnose and manage many infectious diseases. Excellent sensitivity, specificity, and speed have made molecular assays an attractive alternative to culture or enzyme immunoassay methods. Many molecular assays are commercially available and FDA approved. Others, especially those that test for less common analytes, are often laboratory developed. Laboratories also often modify FDA-approved assays to include different extraction systems or additional specimen types. The Clinical Laboratory Improvement Amendments (CLIA) federal regulatory standards require clinical laboratories to establish and document their own performance specifications for laboratory-developed tests to ensure accurate and precise results prior to implementation of the test. The performance characteristics that must be established include accuracy, precision, reportable range, reference interval, analytical sensitivity, and analytical specificity. Clinical laboratories are challenged to understand the requirements and determine the types of experiments and analyses necessary to meet the requirements. A variety of protocols and guidelines are available in various texts and documents. Many of the guidelines are general and more appropriate for assays in chemistry sections of the laboratory but are applied in principle to

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molecular assays. This review presents information that laboratories may consider in their efforts to meet regulatory requirements.

Free full text available from PubMed
PMID: 20610823

D) Role of Molecular Diagnostics in the Management of Infectious Disease Emergencies

Krishna NK, Cunnion KM. Role of molecular diagnostics in the management of infectious disease emergencies. *Med Clin North Am.* 2012 Nov; 96(6): 1067-1078.

Summary: In the setting of infectious disease emergencies, rapid and accurate identification of the causative agent is critical to optimizing antimicrobial therapy in a timely manner. It is clearly evident that the age of molecular diagnostics is now upon us, with real-time PCR becoming the standard of diagnosis for many infectious disease emergencies in either monoplex or multiplex format. Other molecular techniques such as whole or partial genome sequencing, microarrays, broad-range PCR, restriction fragment length polymorphisms, and molecular typing are also being used. However, for most small clinical laboratories, implementation of these advanced molecular techniques is not feasible owing to the high cost of instrumentation and reagents. If these tests are not available in-house, samples can be sent to national reference laboratories (eg, Mayo Medical Laboratories and Quest Diagnostics) for real-time PCR assays that can be completed in 1 day. It is anticipated that over time commercial real-time PCR tests and instrumentation will become more standardized and affordable, allowing individual laboratories to conduct tests locally, thus further reducing turnaround time. Although real-time PCR has been proved to expand our diagnostic capability, it must be stressed that such molecular methodology constitutes only an additional tool in the diagnosis of infectious diseases in emergency situations. Phenotypic methodologies (staining, cultures, biochemical tests, and serology) still play a critical role in identifying, confirming, and providing antibiotic susceptibility testing for many microbial pathogens. As multiplex assays become increasingly available, there will be even greater temptation for taking a "shotgun" approach to diagnostic testing. These new technologies will not substitute for a proper history

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and physical examination leading to a thoughtful differential diagnosis. None the less, these new molecular tests increase the capability of the diagnostician to rapidly identify the microbiological etiology of an infection. An added advantage of rapid diagnostic tests often not emphasized is the capability to rule out certain diagnoses for which unnecessary antimicrobial therapy may otherwise be instituted and/or continued.

Full text available from *Medical Clinics of North America* (USD 31.50)
PMID: 23102477

5.2 Types of Molecular Testing for Infectious Diseases

A) Molecular Methods and Platforms for Infectious Diseases Testing: A Review of FDA-Approved and Cleared Assays
Emmadi R, Boonyaratanakornkit JB, Selvarangan R, et al. Molecular methods and platforms for infectious diseases testing: a review of FDA-approved and cleared assays. *J Mol Diagn.* 2011 Nov; 13(6):583–604.

Summary: The superior sensitivity and specificity associated with the use of molecular assays has greatly improved the field of infectious disease diagnostics by providing clinicians with results that are both accurate and rapidly obtained. Herein, we review molecularly based infectious disease diagnostic tests that are Food and Drug Administration approved or cleared and commercially available in the United States as of December 31, 2010. We describe specific assays and their performance, as stated in the Food and Drug Administration's Summary of Safety and Effectiveness Data or the Office of In Vitro Diagnostic Device Evaluation and Safety's decision summaries, product inserts, or peer-reviewed literature. We summarize indications for testing, limitations, and challenges related to implementation in a clinical laboratory setting for a wide variety of common pathogens. The information presented in this review will be particularly useful for laboratories that plan to implement or expand their molecular offerings in the near term.

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Free full text available from *Journal of Molecular Diagnostics*
PMID: 21871973

B) Real-Time PCR in Clinical Microbiology: Applications for Routine Laboratory Testing

Espy MJ, Uhl JR, Sloan LM, et al. Real-time PCR in clinical microbiology: applications for routine laboratory testing. *Clin Microbiol Rev*. 2006 Jan;19(1):165-256.

Summary: Real-time PCR has revolutionized the way clinical microbiology laboratories diagnose many human microbial infections. This testing method combines PCR chemistry with fluorescent probe detection of amplified product in the same reaction vessel. In general, both PCR and amplified product detection are completed in an hour or less, which is considerably faster than conventional PCR detection methods. Real-time PCR assays provide sensitivity and specificity equivalent to that of conventional PCR combined with Southern blot analysis, and since amplification and detection steps are performed in the same closed vessel, the risk of releasing amplified nucleic acids into the environment is negligible. The combination of excellent sensitivity and specificity, low contamination risk, and speed has made real-time PCR technology an appealing alternative to culture-or immunoassay-based testing methods for diagnosing many infectious diseases. This review focuses on the application of real-time PCR in the clinical microbiology laboratory.

Free full text available from PubMed
PMID: 16418529
NOTE: Also cited in Section 2.3

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5.2.1 Viruses

5.2.1.1 Cytomegalovirus

A) Virologic Suppression Measured by a Cytomegalovirus (CMV) DNA Test Calibrated to the World Health Organization International Standard Is Predictive of CMV Disease Resolution in Transplant Recipients

Razonable RR, Åsberg A, Rollag H, et al. Virologic Suppression Measured by a Cytomegalovirus (CMV) DNA Test Calibrated to the World Health Organization International Standard Is Predictive of CMV Disease Resolution in Transplant Recipients. *Clin Infect Dis.* 2013 Mar 13.

Summary: Cytomegalovirus (CMV) load measurement is used to assess the efficacy of treatment of CMV disease, but lacks standardization. Using the World Health Organization (WHO) international standard for reporting, we correlated viral load with CMV disease resolution. Methods. CMV load was quantified in plasma using a test calibrated to the WHO standard. Three predictive rules were predefined to determine association between CMV DNAemia and outcome: (1) pretreatment CMV DNA of <18 200 (4.3 log10) IU/mL; (2) viral load declines of 1.0, 1.5, 2.0, and 2.5 log10 IU/mL from baseline to days 7, 14, and 21 of treatment, respectively; and (3) viral suppression <137 (2.1 log10) IU/mL at days 7, 14, and 21. Analysis was performed using Cox proportional hazard models. Results. Of 267 patients, 251 had CMV disease resolution by day 49 of treatment. Patients with pretreatment CMV DNA of <18 200 (4.3 log10) IU/mL had faster time to disease resolution (adjusted hazard ratio [AHR], 1.56; P = .001). Patients with CMV load suppression (<137 IU/mL [<2.1 log10]) at days 7, 14, and 21 had faster times to clinical disease resolution (AHRs, 1.61, 1.73, and 1.64, and P = .005, <.001, and <.001, respectively). Relative CMV load reductions from baseline were not significantly associated with faster resolution of CMV disease. Conclusions. Patients with pretreatment CMV DNA of <18 200 (4.3 log10) IU/mL are 1.5 times more likely to have CMV disease resolution. CMV suppression (<137 [2.1 log10] IU/mL), as measured by a test calibrated to the WHO Standard, is predictive of

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clinical response to antiviral treatment. Clinical Trials Registration. NCT00431353.

Free full text available from *Clinical Infectious Diseases*
PMID: 23418272

B) Overview: Cytomegalovirus and the Herpesviruses in Transplantation

Fishman JA. Overview: cytomegalovirus and the herpesviruses in transplantation. *Am J Transplant.* 2013 Feb; 13 Suppl 3(1-8); quiz 8.

Summary: Herpesviruses infect most animal species. Infections due to the eight human herpesviruses (HHV) are exacerbated by immunosuppression in organ transplantation. The special features of the herpesvirus life cycle include the ability to establish latent, nonproductive infection and the life-long capacity for reactivation to productive, lytic infection. Interactions between latent virus and the immune system determine the frequency and severity of symptomatic infections. The immunologic and cellular effects of herpesvirus infections contribute to risk for opportunistic infections and graft rejection. Among the most important advances in transplantation are laboratory assays for the diagnosis and monitoring of herpesvirus infections and antiviral agents with improved efficacy in prophylaxis and therapy. For herpes simplex virus, varicella zoster virus and cytomegalovirus, these advances have significantly reduced the morbidity of infection. The syndromes of EBV-associated posttransplant lymphoproliferative disorders (PTLD) and Kaposi's sarcoma remain important complications of immunosuppression. The epidemiology and essential biology of human herpesvirus is reviewed.

Free full text abstract available from *American Journal of Transplantation*
PMID: 23347210

C) An International Multicenter Performance Analysis of Cytomegalovirus Load Tests

Hirsch HH, Lautenschlager I, Pinsky BA, et al. An international multicenter performance analysis of cytomegalovirus load tests. *Clin Infect Dis.* 2013 Feb; 56(3): 367-373.

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Summary: Quantification of cytomegalovirus (CMV) load is central to the management of CMV infections in immunocompromised patients, but quantitative results currently differ significantly across methods and laboratories. METHODS: The COBAS AmpliPrep/COBAS TaqMan CMV Test (CAP/CTM CMV test), developed using the first World Health Organization CMV standard in the calibration process, was compared to local assays used by 5 laboratories at transplant centers in the United States and Europe. Blinded plasma panels (n = 90) spiked with 2.18-6.7 log(10) copies/mL and clinical plasma samples from immunocompromised patients (n = 660) were tested. RESULTS: Observed mean panel member concentrations by site and 95% confidence intervals (CIs) of the data combined across sites were narrower for CAP/CTM CMV test compared with local assays. The 95% CI in log(10) copies/mL of the combined data per panel member for CAP/CTM CMV test vs comparator assays was .17 vs 1.5 at 2.18 log(10) copies/mL; .14 vs .52 at 2.74 log(10) copies/mL; .16 vs .6 at 3.3 log(10) copies/mL; .2 vs 1.11 at 4.3 log(10) copies/mL; .21 vs 1.13 at 4.7 log(10) copies/mL; and .18 vs 1.4 at 6.7 log(10) copies/mL. In clinical specimens, constant and variable quantification differences between the CAP/CTM CMV test and comparator assays were observed. CONCLUSIONS: High interlaboratory agreement and precision of CAP/CTM CMV test results across 5 different laboratories over 4 orders of magnitude suggest that this assay could be valuable in prospective studies identifying clinical viral load thresholds for CMV treatment.

Free full text available from PubMed
PMID: 23097587

5.2.1.2 Enterovirus

A) Diagnosis and Outcomes of Enterovirus Infections in Young Infants

Rittichier KR, Bryan PA, Bassett KE, et al. Diagnosis and outcomes of enterovirus infections in young infants. *Pediatr Infect Dis J.* 2005 Jun; 24(6): 546-550.

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Summary: Enterovirus (EV) infections commonly cause fever in infants younger than 90 days of age. The polymerase chain reaction (PCR) has improved our ability to diagnose EV infections. OBJECTIVE: To evaluate the utility of blood and cerebrospinal fluid (CSF) specimens for the diagnosis of EV infections by PCR and to describe a large cohort of EV-infected infants. DESIGN/METHODS: Febrile infants younger than 90 days of age evaluated for sepsis at Primary Children's Medical Center in Salt Lake City, UT, were enrolled in a prospective study designed to identify viral infections from December 1996 to June 2002. All patients had bacterial cultures of blood, urine and CSF. Testing for EV was performed by PCR and/or viral cultures. Patients who were positive for EV were identified for this study. RESULTS: Of 1779 febrile infants enrolled, 1061 had EV testing and 214 (20%) were EV-positive. EV infections were diagnosed by PCR of blood, CSF or both in 93% of infants. PCR testing was positive in blood in 57%, and blood was the only positive specimen for 22% of EV infected infants. PCR of CSF was positive in 74%. The mean age of infants with EV infection was 33 days, with 18% younger than 14 days and 5% younger than 7 days. Fifty percent of EV-positive infants had CSF pleocytosis. Of EV PCR-positive infants, 91% were admitted, and 2% required intensive care. Possible serious EV disease was diagnosed in <1%, and there were no deaths. Twelve infants (5.6%) had concomitant urinary tract infection, and 3 (1%) had bacteremia. CONCLUSIONS: EV infections are common in febrile infants younger than 90 days. Blood and CSF are equally likely to yield positive results by PCR, but the combination of both specimens improved the diagnostic yield.

Full text available from *Pediatric Infectious Disease Journal* (subscription required)
PMID: 15933567

B) Reverse-transcription Polymerase Chain Reaction Detection of the Enteroviruses

Romero JR. Reverse-transcription polymerase chain reaction detection of the enteroviruses. *Arch Pathol Lab Med*. 1999 Dec; 123(12):1161-1169.

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Summary: This review focuses on commercial and in-house-developed reverse-transcription polymerase chain reaction (RT-PCR) assays used for the detection of enteroviral infections. In addition to providing details on the performance of RT-PCR, its specificity, and sensitivity, the clinical utility of this diagnostic method with specific reference to its impact on hospitalization and cost savings is addressed. DATA SOURCES: MEDLINE was searched for reports relating to RT-PCR detection of the enteroviruses in adults and children. The search was restricted to studies reported in English language journals. STUDY SELECTION: Reports documenting detailed information regarding the RT-PCR conditions, primers, sensitivity, specificity and, if relevant, clinical impact were selected for analysis. DATA EXTRACTION: Details regarding method of extraction of the enteroviral genome, the primers used, RT-PCR conditions, and sensitivity and specificity of the assay were extracted from the literature. For reports detailing the use of RT-PCR in the clinical management of enteroviral infections in children, the reduction in duration of hospitalization and health care cost savings were recorded. DATA SYNTHESIS: Reverse-transcription PCR can increase the yield of detection of enteroviruses from cerebrospinal fluid by a mean of approximately 20% over tissue culture. Reverse-transcription PCR of cerebrospinal fluid has been shown to exhibit sensitivity and specificity values of 86% to 100% and 92% to 100%, respectively. Reductions of 1 to 3 days of hospitalization per patient are predicted if RT-PCR is used to diagnose enteroviral meningitis in children. CONCLUSIONS: Reverse-transcription PCR detection of enteroviral infections is an extremely rapid, sensitive, and specific diagnostic modality. Both commercial assays and assays developed in-house appear to be equivalent with regard to sensitivity and specificity. Reverse-transcription PCR diagnosis of enteroviral infections in children could reduce the length of hospitalization and result in significant health care cost savings.

Free full text available from the CAP's *Archives*
PMID: 10583920

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5.2.1.3 Epstein Barr Virus

A) Determining EBV Load: Current Best Practice and Future Requirements

Ruf S, Wagner HJ. Determining EBV load: current best practice and future requirements. *Expert Rev Clin Immunol.* 2013 Feb; 9(2): 139-151.

Summary: EBV, a gammaherpesvirus and the pathogenic agent for infectious mononucleosis, is also associated with a broad spectrum of lymphoid and epithelial malignancies in immunocompetent and immunosuppressed individuals. EBV-DNA-load measurement by PCR has been shown to be a potential tool for the diagnosis of these diseases, a prognostic factor of their outcome and a successful method to monitor immunosuppressed patients. Since the end of 2011, there is an international WHO standard reference for EBV quantification available; however, many questions still remain; for instance about the optimal amplified region of the EBV genome, or the best-used specimen for EBV detection. Additionally, the optimal specimen and amplified region may vary in different malignancies. In this article, the authors review the different methods to measure EBV load, focus on the best-used specimen for the different EBV-associated malignancies and discuss future requirements and opportunities for EBV-load measurement.

Full text available from Expert Review of Clinical Immunology (USD 86.00)
PMID: 23390945

B) Comparison of Six Different Specimen Types for Epstein-Barr Viral Load Quantification in Peripheral Blood of Pediatric Patients After Heart Transplantation or After Allogeneic Hematopoietic Stem Cell Transplantation

Ruf S, Behnke-Hall K, Gruhn B, et al. Comparison of six different specimen types for Epstein-Barr viral load quantification in peripheral blood of pediatric patients after heart transplantation or after allogeneic hematopoietic stem cell transplantation. *J Clin Virol.* 2012 Mar; 53(3): 186-194.

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Summary: Epstein-Barr Virus (EBV) a gamma-herpes virus is associated with a spectrum of lymphoid and epithelial malignancies including posttransplant lymphoproliferative disorders (PTLD). EBV-load measurement has been shown to be important for the monitoring of these patients. However, in contrast to the viral quantification of human immunodeficiency virus or human hepatitis C virus, the EBV-load measurement has not been completely standardized as yet. OBJECTIVES: In this study, we compared the EBV DNA levels in whole blood (WB), plasma, peripheral mononuclear cells (PBMC) and B-cells (BC) in children and adolescents after heart transplantations (HTx) and allogeneic hematopoietic stem cell transplantations (HSCT). STUDY DESIGN: In a period of 2 years (from May 2007 to May 2009) we collected 547 samples of 96 cardiac transplant recipients and 248 samples of 37 patients who underwent HSCT. For EBV DNA quantification we used a duplex real-time PCR (ABI Prism 7500, Applied Biosystems). Additionally, EBV-load of PBMC and BC were normalized with respect to endogenous cell DNA. RESULTS: In both patient populations we found no significant difference of test sensitivity for the EBV detection. In PBMC as well as BC, there was a high correlation between the analysis of cells with and without normalization in both populations. Spearman's correlation coefficient rho between PBMC without and PBMC with normalization was rho=0.98 (P<0.0001) in patients after HTx and rho=0.99 (P<0.0001) in patients after HSCT. Correlation between BC with and without normalization was rho=0.98 (P<0.0001) in patients after HTx and rho=0.995 (P<0.0001) in patients after HSCT. When comparing the different blood compartments for EBV quantification in both populations, the strongest correlations were found between the EBV DNA levels in WB and PBMC (HTx: rho=0.93, P<0.0001; HSCT: rho=0.81, P<0.0001) followed by PBMC and BC (HTx: rho=0.87, P<0.0001; HSCT: rho=0.81, P<0.0001) as well as WB and BC (HTx: rho=0.86, P<0.0001; HSCT: rho=0.75, P<0.0001). In contrast, the correlation coefficients between plasma and the other blood compartments (WB as well as PBMC or BC) were lower. Six patients developed seven episodes of PTLD (five patients after HTx and one after renal transplantation). Analyzing the different blood compartments, we found that a threshold of WB >/=20,000EBV-copies/ml and plasma >/=1000EBV-copies/ml had the highest sensitivities and specificities (WB: sensitivity 100%, specificity 87%

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and plasma: sensitivity 88%, specificity 98%). CONCLUSION: Normalization towards an endogenous control does not seem to be necessary for EBV quantification in peripheral blood. The analysis of whole blood correlates well with B-cells and PBMC. Routine screening of EBV DNA in whole blood appeared to be a useful tool supplemented by EBV-load measurement in plasma to discriminate chronic high EBV-load carrier without risk for PTLD from those who are at risk for PTLD. Values in whole blood higher than 20,000EBV-copies/ml WB and plasma values higher than 1000EBV-copies/ml plasma indicated PTLD in our series.

Full text available from *Journal of Clinical Virology* (USD 31.50)
PMID: 22182950

C) Interlaboratory Comparison of Epstein-Barr Virus Viral Load Assays

Preiksaitis JK, Pang XL, Fox JD, Fenton JM, Caliendo AM, Miller GG; American Society of Transplantation Infectious Diseases Community of Practice. Interlaboratory comparison of epstein-barr virus viral load assays. *Am J Transplant.* 2009 Feb; 9(2): 269-279.

Summary: To assess interlaboratory variability in qualitative and quantitative Epstein-Barr virus (EBV) viral load (VL) testing, we distributed a panel of samples to 28 laboratories in the USA, Canada and Europe who performed testing using commercially available reagents (n = 12) or laboratory-developed assays (n = 18). The panel included two negatives, seven constructed samples using Namalwa and Molt-3 cell lines diluted in plasma (1.30-5.30 log(10) copies/mL) and three clinical plasma samples. Significant interlaboratory variation was observed for both actual (range 1.30-4.30 log(10) copies/mL) and self-reported (range, 1.70-3.30 log(10) copies/mL) lower limits of detection. The variation observed in reported results on individual samples ranged from 2.28 log(10) (minimum) to 4.14 log(10) (maximum). Variation was independent of dynamic range and use of commercial versus laboratory-developed assays. Overall, only 47.0% of all results fell within acceptable standards of variation: defined as the expected result +/- 0.50 log(10). Interlaboratory variability on replicate samples was significantly greater than intralaboratory variability ($p < 0.0001$). Kinetics of change in VL appears more

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relevant than absolute values and clinicians should understand the uncertainty associated with absolute VL values at their institutions. The creation of an international reference standard for EBV VL assay calibration would be an initial important step in quality improvement of this laboratory tool.

Free full text available from *American Journal of Transplantation*
PMID: 19178414

D) Epstein-Barr Virus Infection and Posttransplant Lymphoproliferative Disorder

Green M, Michaels MG. Epstein-Barr virus infection and posttransplant lymphoproliferative disorder. *Am J Transplant.* 2013 Feb; 13 Suppl 3:41-54; quiz 54.

Summary: Epstein-Barr virus (EBV) is an important pathogen in recipients of solid organ transplants (SOT). Infection with EBV manifests as a spectrum of diseases/malignancies ranging from asymptomatic viremia through infectious mononucleosis to posttransplant lymphoproliferative disorder (PTLD). EBV disease and its associated PTLD is more frequently seen when primary EBV infection occurs after transplant, a common scenario in pediatric SOT recipients. Intensity of immunosuppressive therapies also influences the risk for PTLD. The use of EBV viral load monitoring facilitates the diagnosis and management of EBV/PTLD as well as being used to inform preemptive therapy with reduction of immunosuppression, the most effective intervention for prevention of and treatment for PTLD. Other therapies, including the rituximab (anti-CD20 monoclonal antibody) and traditional chemotherapy, are also useful in the treatment of established PTLD. The future development of standards for management based on EBV viral load and routine monitoring of EBV-specific CTL responses promise further improvement in outcomes with EBV and PTLD.

Free full text abstract available from *American Journal of Transplantation*
PMID: 23347213

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5.2.1.4 Hepatitis C Virus

A) Testing for HCV Infection: An Update of Guidance for Clinicians and Laboratorians

Centers for Disease Control and Prevention. Testing for HCV Infection: An Update of Guidance for Clinicians and Laboratorians. *MMWR. Morbidity and mortality weekly report*. 2013;62(18):362-365.

Summary: In the United States, an estimated 4.1 million persons have been infected with hepatitis C virus (HCV), of whom an estimated 3.2 (95% confidence interval [CI] = 2.7–3.9) million are living with the infection (*1*). New infections continue to be reported particularly among persons who inject drugs and persons exposed to HCV-contaminated blood in health-care settings with inadequate infection control (*2*). Since 1998, CDC has recommended HCV testing for persons with risks for HCV infection (*3*). In 2003, CDC published guidelines for the laboratory testing and result reporting of antibody to HCV (*4*). In 2012, CDC amended testing recommendations to include one-time HCV testing for all persons born during 1945–1965 regardless of other risk factors (*1*). CDC is issuing this update in guidance because of 1) changes in the availability of certain commercial HCV antibody tests, 2) evidence that many persons who are identified as reactive by an HCV antibody test might not subsequently be evaluated to determine if they have current HCV infection (*5*), and 3) significant advances in the development of antiviral agents with improved efficacy against HCV (*6*). Although previous guidance has focused on strategies to detect and confirm HCV antibody (*3,4*), reactive results from HCV antibody testing cannot distinguish between persons whose past HCV infection has resolved and those who are currently HCV infected. Persons with current infection who are not identified as currently infected will not receive appropriate preventive services, clinical evaluation, and medical treatment. Testing strategies must ensure the identification of those persons with current HCV infection. This guidance was written by a workgroup convened by CDC and the Association of Public Health Laboratories (APHL), comprising experts from CDC, APHL, state and local public health departments, and academic and independent diagnostic testing laboratories, in consultation with experts from the Veterans Health Administration and the Food and Drug Administration

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(FDA). The workgroup reviewed laboratory capacities and practices relating to HCV testing, data presented at the CDC 2011 symposium on identification, screening and surveillance of HCV infection (*7*), and data from published scientific literature on HCV testing. Unpublished data from the American Red Cross on validation of HCV antibody testing also were reviewed.

Free full text available from *Morbidity and Mortality Weekly Report*

B) The Role of Resistance in HCV Treatment

Vermehren J, Sarrazin C. The role of resistance in HCV treatment. *Best Pract Res Clin Gastroenterol.* 2012 Aug; 26(4): 487-503.

Summary: The recent development of small molecule compounds that directly inhibit the viral life cycle represents a major milestone for the treatment of chronic hepatitis C virus (HCV) infection. These new drugs that are collectively termed direct-acting antivirals (DAA) include a range of inhibitors of the non-structural (NS) 3/4A protease, NS5B polymerase and NS5A protein. Two NS3/4A protease inhibitors (boceprevir and telaprevir) in combination with pegylated interferon and ribavirin have now been approved for the treatment of chronic HCV genotype 1 infection and cure rates could be increased by 20-30%. However, the majority of DAAs is still in early clinical development. The rapid replication rate of HCV, along with the error-prone polymerase activity leads to a high genetic diversity among HCV virions that includes mutants with reduced susceptibility to DAA-therapy. These resistance-associated variants often occur at very low frequencies. However, during DAA-based treatment, rapid selection of resistance mutations may occur, eventually leading to viral break-through. A number of variants with different levels of resistance have been described in vitro and in vivo for virtually all DAAs. We review the parameters that determine DAA resistance as well as the clinical implications of resistance testing. In addition, the most recent literature and conference data on resistance profiles of DAAs in clinical development and future strategies to avoid the emergence of viral resistance are also discussed.

Full text available from *Best Practice & Research Clinical Gastroenterology* (USD 31.50)

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PMID: 23199507

C) Recommendations for the Identification of Chronic Hepatitis C Virus Infection Among Persons Born During 1945-1965

Smith BD, Morgan RL, Beckett GA, et al. Recommendations for the identification of chronic hepatitis C virus infection among persons born during 1945-1965. *MMWR Recomm Rep.* 2012 Aug 17; 61(RR-4): 1-32.

Summary: Hepatitis C virus (HCV) is an increasing cause of morbidity and mortality in the United States. Many of the 2.7-3.9 million persons living with HCV infection are unaware they are infected and do not receive care (e.g., education, counseling, and medical monitoring) and treatment. CDC estimates that although persons born during 1945-1965 comprise an estimated 27% of the population, they account for approximately three fourths of all HCV infections in the United States, 73% of HCV-associated mortality, and are at greatest risk for hepatocellular carcinoma and other HCV-related liver disease. With the advent of new therapies that can halt disease progression and provide a virologic cure (i.e., sustained viral clearance following completion of treatment) in most persons, targeted testing and linkage to care for infected persons in this birth cohort is expected to reduce HCV-related morbidity and mortality. CDC is augmenting previous recommendations for HCV testing (CDC. Recommendations for prevention and control of hepatitis C virus (HCV) infection and HCV-related chronic disease. MMWR 1998;47[No. RR-19]) to recommend one-time testing without prior ascertainment of HCV risk for persons born during 1945-1965, a population with a disproportionately high prevalence of HCV infection and related disease. Persons identified as having HCV infection should receive a brief screening for alcohol use and intervention as clinically indicated, followed by referral to appropriate care for HCV infection and related conditions. These recommendations do not replace previous guidelines for HCV testing that are based on known risk factors and clinical indications. Rather, they define an additional target population for testing: persons born during 1945-1965. CDC developed these recommendations with the assistance of a work group representing diverse expertise and perspectives. The recommendations are informed by the Grading of Recommendations

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Assessment, Development, and Evaluation (GRADE) framework, an approach that provides guidance and tools to define the research questions, conduct the systematic review, assess the overall quality of the evidence, and determine strength of the recommendations. This report is intended to serve as a resource for health-care professionals, public health officials, and organizations involved in the development, implementation, and evaluation of prevention and clinical services. These recommendations will be reviewed every 5 years and updated to include advances in the published evidence.

Free full text available from *Morbidity and Mortality Weekly Report*

D) Genetics of IL28B and HCV--Response to Infection and Treatment

Hayes CN, Imamura M, Aikata H, Chayama K. Genetics of IL28B and HCV--response to infection and treatment. *Nat Rev Gastroenterol Hepatol.* 2012 Jul; 9(7): 406-417.

Summary: The IL28B locus attracted the attention of HCV researchers after a series of genome-wide association studies independently identified a strong association between common IL28B polymorphisms and the outcome of PEG-IFN-alpha plus ribavirin combination therapy in patients chronically infected with HCV genotype 1. This association was subsequently replicated for other HCV genotypes and has been linked to spontaneous eradication of HCV, development of steatosis and biochemical changes (such as altered levels of gamma-glutamyl transpeptidase and LDL). Despite the introduction of direct-acting antiviral drugs, IL28B genetics are likely to play a part in patient selection and treatment decisions-moving towards a personalized approach to therapy. In HCV-infected patients with the so-called favourable IL28B genotype (rs12979860 CC; associated with better treatment response), hepatic expression levels of IL28B and interferon-stimulated genes seem to be reduced at baseline, but are induced more strongly after IFN-alpha administration, perhaps resulting in more effective elimination of the virus. Clarification of the mechanisms underlying these biological phenomena will lead to improved understanding of the antiviral effects of IFN-lambda and, ideally, to the development of better therapies against HCV infection. This Review summarizes current

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understanding of the role of IL28B in HCV infection and response to therapy.

Full text available from *Nature Reviews Gastroenterology and Hepatology* (USD 32.00)
PMID: 22641049
NOTE: Also cited in Section 6

5.2.1.5 Human Immunodeficiency Virus (HIV)

A) Routine HIV Testing, Public Health, and the USPSTF--An End to the Debate

Bayer R, Oppenheimer GM. Routine HIV testing, public health, and the USPSTF--an end to the debate. *N Engl J Med.* 2013 Mar 7; 368(10): 881-884.

Summary: The U.S. Preventive Services Task Force (USPSTF) is poised to release recommendations on screening for human immunodeficiency virus (HIV) infection that will endorse the routine testing of adults and adolescents, a position first adopted by the Centers for Disease Control and Prevention (CDC) in 2006. Based on an exacting systematic examination of the new evidence on clinical and public health benefits of early identification of HIV infection that has emerged since 2005, when the initial USPSTF review led to rejection of routine screening, the new recommendations will be a critical guide to clinical practice. They will also carry important policy implications, since the Affordable Care Act (ACA) mandates that all public and private health plans provide coverage for USPSTF-recommended preventive services without patient copayments.

Free full text available from *New England Journal of Medicine*
PMID: 23425134

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5.2.1.6 Respiratory Viruses

A) Molecular Diagnosis of Respiratory Virus Infections

Mahony JB, Petrich A, Smieja M. Molecular diagnosis of respiratory virus infections. *Crit Rev Clin Lab Sci*. 2011 Sep-Dec;48(5-6):217-49.

Summary: The appearance of eight new respiratory viruses, including the SARS coronavirus in 2003 and swine-origin influenza A/H1N1 in 2009, in the human population in the past nine years has tested the ability of virology laboratories to develop diagnostic tests to identify these viruses. Nucleic acid based amplification tests (NATs) for respiratory viruses were first introduced two decades ago and today are utilized for the detection of both conventional and emerging viruses. These tests are more sensitive than other diagnostic approaches, including virus isolation in cell culture, shell vial culture (SVC), antigen detection by direct fluorescent antibody (DFA) staining, and rapid enzyme immunoassay (EIA), and now form the backbone of clinical virology laboratory testing around the world. NATs not only provide fast, accurate and sensitive detection of respiratory viruses in clinical specimens but also have increased our understanding of the epidemiology of both new emerging viruses such as the pandemic H1N1 influenza virus of 2009, and conventional viruses such as the common cold viruses, including rhinovirus and coronavirus. Multiplex polymerase chain reaction (PCR) assays introduced in the last five years detect up to 19 different viruses in a single test. Several multiplex PCR tests are now commercially available and tests are working their way into clinical laboratories. The final chapter in the evolution of respiratory virus diagnostics has been the addition of allelic discrimination and detection of single nucleotide polymorphisms associated with antiviral resistance. These assays are now being multiplexed with primary detection and subtyping assays, especially in the case of influenza virus. These resistance assays, together with viral load assays, will enable clinical laboratories to provide physicians with new and important information for optimal treatment of respiratory virus infections.

Full text available from *Critical Reviews in Clinical Laboratory Science* (USD 50.00 for 24 hour access)
PMID: 22185616

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B) Multiplex RVPs Enticing Labs to Molecular

Paxton A. Multiplex RVPs enticing labs to molecular. *CAP TODAY.* 2010 April.

Summary: Multiplex respiratory virus panels, which use PCR technology to detect up to 20 or 30 pathogens in one swoop, may not be able to pinpoint the virus infecting every patient with respiratory symptoms. But they're getting close.

Free full text available from *CAP TODAY*

C) For Respiratory Virus Detection, a Golden Age

Check W. For Respiratory Virus Detection, A Golden Age. *CAP Today.* 2012 April.

Summary: When it comes to molecular deteection of respiratory viruses, laboratorians today have an embarrassment of riches. Dr. Christine Ginnochio presented a review of multianalyte platforms for detecting respiratory viruses. With so many platforms avavilable--Dr. Ginocchio discussed 13, seven cleareed and six in trials--and with such a wide range in multiplexity, turnaround time, throughput, degree of automation, and cost, how is a lab to know which system best fits its needs? Or, for that matter, whether it even needs a multiplex platform?

Free full text available from *CAP TODAY*

D) Strengths and Weaknesses of FDA-Approved/Cleared Diagnostic Devices for the Molecular Detection of Respiratory Pathogens

Ginocchio CC. Strengths and weaknesses of FDA-approved/cleared diagnostic devices for the molecular detection of respiratory pathogens. *Clin Infect Dis.* 2011 May; 52 Suppl 4:S312-325.

Summary: The rapid, sensitive, and specific identification of the microbial etiological characteristics of respiratory tract infections enhances the appropriate use of both antibiotics and antiviral agents and reduces the risk of nosocomial transmission. This article reviews the current nucleic acid amplification tests approved by the U.S. Food

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and Drug Administration (FDA) for the detection of respiratory pathogens. In addition, Emergency Use Authorization tests for the detection of 2009 influenza A H1N1 are discussed. The advantages and limitations of the current FDA-approved/cleared tests are reviewed.

Free full text available from *Clinical Infectious Diseases*
PMID: 21460290

5.2.2 Bacteria

5.2.2.1 Chlamydia Trachomatis and Neisseria Gonorrhoeae

A) Laboratory Diagnostic Testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae:* Expert Consultation Meeting Summary Report

Association of Public Health Laboratories. Laboratory Diagnostic Testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae:* Expert Consultation Meeting Summary Report; January 13-15, 2009; Atlanta, GA: Association of Public Health Laboratories in cooperation with the Centers for Disease Control.

Summary: In the last decade there have been major changes and improvements in STD testing technologies. While these changes have created great opportunities for more rapid and accurate STD diagnosis, they may also create confusion when laboratories attempt to incorporate new technologies into the existing structure of their laboratory. With this in mind, the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL) convened an expert panel to evaluate available information and produce recommendations for inclusion in the Guidelines for the Laboratory Diagnosis of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in the United States. An in-person meeting to formulate these recommendations was held on January 13-15, 2009 on the CDC Roybal campus. The panel included public health laboratorians, STD researchers, STD clinicians, STD Program Directors and other

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STD program staff. Representatives from the Food and Drug Administration (FDA) and Centers for Medicare & Medicaid Services (CMS) were also in attendance. The target audience for these recommendations includes laboratory directors, laboratory staff, microbiologists, clinicians, epidemiologists, and disease control personnel. For several months prior to the in-person consultation, these workgroups developed key questions and researched the current literature to ensure that any recommendations made were relevant and evidence based. Published studies compiled in Tables of Evidence provided a framework for group discussion addressing several key questions.

Free full text available from Association of Public Health Laboratories

B) The Swedish New Variant of Chlamydia Trachomatis (nvCT) Remains Undetected by Many European Laboratories as Revealed in the Recent PCR/NAT Ring Trial Organized by INSTAND e.V., Germany

Reischl U, Straube E, Unemo M. The Swedish new variant of Chlamydia trachomatis (nvCT) remains undetected by many European laboratories as revealed in the recent PCR/NAT ring trial organised by INSTAND e.V., Germany. *Euro Surveill*. 2009 Aug; 14(32):pii:19302.

Summary: The May 2009 round of INSTAND's ring trial "Chlamydia trachomatis detection PCR/NAT" included a sample with high amount of the Swedish new variant of C. trachomatis (nvCT). A spectrum of at least 12 different commercial diagnostic nucleic acid amplification tests (NAATs) and many different in house NAATs were applied by the 128 participating laboratories which reported 152 results. Approximately 80% of the results correctly reported the presence of C. trachomatis in the nvCT specimen. The nvCT sample was mainly missed, as expected, by participants using the Roche COBAS Amplicor CT/NG (15.5% of reported results) but also by several participants using in house NAATs. The trend towards using nvCT-detecting NAATs is obvious and in addition to the new dual-target NAATs from Roche and Abbott, and BD ProbeTec ET, also a number of new CE mark-certified commercial tests from smaller diagnostic companies as well as many different in house NAATs were used.

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Laboratories using commercial or in house NAATs that do not detect the nvCT are encouraged to carefully monitor their C. trachomatis incidence, participate in appropriate external quality assurance and controls schemes, and consider altering their testing system. The reliable detection of low amounts of the wildtype C. trachomatis strain in other samples of the ring trial set indicates a good diagnostic performance of all applied commercial NAATs while also detecting the nvCT strain.

Free full text available from *Eurosurveillance*
PMID: 19679035

5.2.2.2 Clostridium Difficile

A) Tests for the Diagnosis of Clostridium Difficile Infection: The Next Generation

Carroll KC. Tests for the diagnosis of Clostridium difficile infection: the next generation. *Anaerobe.* 2011 Aug; 17(4): 170-174.

Summary: Clostridium difficile (C. difficile) causes 25-30% of cases of antibiotic associated diarrhea and most cases of pseudomembranous colitis. Patients presenting with diarrhea after hospitalization for 3 or more days should be tested for C. difficile. There are many options available for testing, each of which has inherent advantages and disadvantages. Most laboratories perform toxin testing using an enzyme immunoassay method. In general these tests have sensitivities ranging from 60 to 70% and specificities of 98%. When using these methods, symptomatic patients with negative tests should be tested by another more sensitive method. Until recently, cell culture cytotoxicity neutralization assays (CCNAs) were considered the gold standard in the U.S. A two-step algorithm using an EIA for glutamate dehydrogenase detection followed by testing positives using CCNA, offered an improved alternative until the availability of molecular assays. Although early studies that compared the GDH assay to CCNA demonstrated high sensitivity and negative predictive values, more recent comparisons to toxigenic culture and PCR have shown the sensitivity to be in the mid to high 80's. When testing using a sensitive assay, repeat testing is not cost-effective. Outbreaks

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caused by a toxin variant epidemic strain have renewed interest in bacterial culture. Toxigenic culture has emerged as the new gold standard against which newer assays should be compared. However, there is no agreed upon standard method for culture performance. At least 4 FDA cleared nucleic acid amplification assays are available to clinical laboratories and several of these have been well evaluated in the literature. Because these assays detect a gene that encodes toxin and not the toxin itself it is important that laboratories test only patients with diarrhea. These molecular assays have been shown to be superior to toxin EIAs, CCNA and 2-step algorithms, but not to toxigenic culture. More studies are needed to assess the impact of molecular tests on treatment and nosocomial spread of Clostridium difficile infections.

Full text available from *Anaerobe* (USD 31.50)
PMID**:** 21376826

5.2.2.3 MRSA (Methicillin-Resistant Staphylococcus Areus)

A) Multicenter Evaluation of the LightCycler MRSA Advanced Test, the Xpert MRSA Assay, and MRSASelect Directly Plated Culture with Simulated Workflow Comparison for the Detection of Methicillin-Resistant Staphylococcus Aureus in Nasal Swabs
Arcenas RC, Spadoni S, Mohammad A, et al. Multicenter evaluation of the LightCycler MRSA advanced test, the Xpert MRSA Assay, and MRSASelect directly plated culture with simulated workflow comparison for the detection of methicillin-resistant Staphylococcus aureus in nasal swabs. *J Mol Diagn*. 2012 Jul;14(4): 367-375.

Summary: Rapid detection of nasal colonization with methicillin-resistant Staphylococcus aureus (MRSA) followed by appropriate infection control procedures reduces MRSA infection and transmission. We compared the performance and workflow of two Food and Drug Administration-approved nucleic acid amplification assays, the LightCycler MRSA Advanced Test and the Xpert MRSA test, with those of directly plated culture (MRSASelect) using 1202 nasal swabs collected at three U.S. sites. The sensitivity of the

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LightCycler test (95.2%; 95% CI, 89.1% to 98.4%) and Xpert assay (99%; 95% CI, 94.8% to 100%) did not differ compared with that of culture; the specificity of the two assays was identical (95.5%; 95% CI, 94.1% to 96.7%) compared with culture. However, sequencing performed on 71 samples with discordant results among the three methods confirmed the presence of MRSA in 40% of samples that were positive by both molecular methods but negative by culture. Workflow analysis from all sites including batch runs revealed average hands-on sample preparation times of 1.40, 2.35, and 1.44 minutes per sample for the LightCycler, Xpert, and MRSASelect methods, respectively. Discrete event simulation analysis of workflow efficiencies revealed that the LightCycler test used less hands-on time for the assay when greater than eight batched samples were run. The high sensitivity and specificity, low hands-on time, and efficiency gains using batching capabilities make the LightCycler test suitable for rapid batch screening of MRSA colonization.

Full text available from *Journal of Molecular Diagnostics* (USD 31.50)
PMID: 22584139

B) Cost of Screening Intensive Care Unit Patients for Methicillin-Resistant Staphylococcus Aureus in Hospitals

Nyman JA, Lees CH, Bockstedt LA, et al. Cost of screening intensive care unit patients for methicillin-resistant Staphylococcus aureus in hospitals. *Am J Infect Control.* 2011 Feb; 39(1): 27-34.

Summary: The objective of this study is to determine the costs per hospital admission of screening intensive care unit patients for methicillin-resistant Staphylococcus aureus (MRSA) and isolating those who are colonized. METHODS: Data on the costs of the intervention come from the Minneapolis Veterans Affairs Medical Center, a 279-bed teaching hospital and outpatient facility. A microcosting approach is used to determine the intervention costs for 3 different laboratory testing protocols. The costs of caring for MRSA-infected patients come from the experience of 241 Minneapolis Veterans Affairs Medical Center patients with MRSA infections in 2004 through 2006. The effectiveness of the intervention comes from the extant literature. To capture the effect of screening on reducing transmission of MRSA to other patients and its effect on costs, a

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Markov simulation model was employed. RESULTS: The intervention was cost saving compared with no intervention for all 3 laboratory processes evaluated and for all of the 1-way sensitivity analyses considered. CONCLUSION: Because of the high cost of caring for a MRSA patient, interventions that reduce the spread of infections-such as screening intensive care unit patients upon admission studied here-are likely to pay for themselves.

Full text available from *American Journal of Infection Control* (USD 14.00)
PMID: 21281884

C) To Screen or Not to Screen for Methicillin-Resistant Staphylococcus Aureus

Peterson LR, Diekema DJ. To screen or not to screen for methicillin-resistant Staphylococcus aureus. *J Clin Microbiol.* 2010 Mar; 48(3): 683-689.

Summary: There are few more compelling questions in clinical microbiology today than the issue of whether or not to screen for the presence of methicillin-resistant Staphylococcus aureus (MRSA), with the results being used to institute infection control interventions aimed at preventing transmission of MRSA in health care environments. Numerous different matters must be addressed when considering a screening program. Who is to be screened, what method is to be employed to detect MRSA, and what sites should be sampled? When and how often should the screening be performed? Who is going to pay for the screening, and, finally and perhaps most importantly, how are screening results to be communicated to health care providers and what kind of interventions are best undertaken based on the results? Numerous governmental agencies have mandated MRSA screening programs, and yet several authorities in infection control organizations have questioned the appropriateness of mandated screening. In this Point-Counterpoint feature, Dr. Lance Peterson of Evanston Hospital (Evanston, IL) offers his perspective on why screening for MRSA is to be encouraged. Dr. Daniel Diekema of the University of Iowa Carver College of Medicine (Iowa City, IA) offers an opposing view.

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PMID: 20071548

D) Assessment of the Influence of Test Characteristics on the Clinical and Cost Impacts of Methicillin-Resistant Staphylococcus Aureus Screening Programs in US Hospitals
Olchanski N, Mathews C, Fusfeld L, Jarvis W. Assessment of the influence of test characteristics on the clinical and cost impacts of methicillin-resistant Staphylococcus aureus screening programs in US hospitals. *Infect Control Hosp Epidemiol.* 2011 Mar; 32(3): 250-257.

Summary: To compare the impacts of different methicillin-resistant Staphylococcus aureus (MRSA) screening test options (eg, polymerase chain reaction [PCR], rapid culture) and program characteristics on the clinical outcomes and budget of a typical US hospital. METHODS: We developed an Excel-based decision-analytic model, using published literature to calculate and compare hospital costs and MRSA infection rates for PCR- or culture-based MRSA screening and then used multivariate sensitivity analysis to evaluate key variables. Same-day PCR testing for a representative 370-bed teaching hospital in the United States was assessed in different populations (high-risk patients, intensive care unit [ICU] patients, or all patients) and compared with other test options. RESULTS: Different screening program populations (all patients, high-risk patients, ICU patients, or patients with previous MRSA colonization or infection only) represented a potential savings of $12,158-$76,624 per month over no program ($188,618). Analysis of multiple test options in high-risk population screening indicated that same-day PCR testing of high-risk patients resulted in fewer infections over 1,720 patient-days (2.9, compared with 3.5 for culture on selective media and 3.8 for culture on nonselective media) and the lowest total cost ($112,012). The costs of other testing approaches ranged from $113,742 to $123,065. Sensitivity analysis revealed that variations in transmission rate, conversion to infection, prevalence increases, and hospital size are important to determine program impact. Among test characteristics, turnaround time is highly influential. CONCLUSION: All screening options showed reductions in infection rates and cost impact improvement over no screening program. Among the options, same-day PCR testing for high-risk patients slightly edges out the

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others in terms of fewest infections and greatest potential cost savings.

Free full text available from *Infection Control and Epidemiology*
PMID: 21460510

E) Costs and Benefits of Rapid Screening of Methicillin-Resistant Staphylococcus Aureus Carriage in Intensive Care Units: A Prospective Multicenter Study

Wassenberg M, Kluytmans J, Erdkamp S, et al. Costs and benefits of rapid screening of methicillin-resistant Staphylococcus aureus carriage in intensive care units: a prospective multicenter study. *Crit Care.* 2012 Feb; 16(1):R22.

Summary: Pre-emptive isolation of suspected methicillin-resistant Staphylococcus aureus (MRSA) carriers is a cornerstone of successful MRSA control policies. Implementation of such strategies is hampered when using conventional cultures with diagnostic delays of three to five days, as many non-carriers remain unnecessarily isolated. Rapid diagnostic testing (RDT) reduces the amount of unnecessary isolation days, but costs and benefits have not been accurately determined in intensive care units (ICUs). METHODS: Embedded in a multi-center hospital-wide study in 12 Dutch hospitals we quantified cost per isolation day avoided using RDT for MRSA, added to conventional cultures, in ICUs. BD GeneOhm MRSA PCR (IDI) and Xpert MRSA (GeneXpert) were subsequently used during 17 and 14 months, and their test characteristics were calculated with conventional culture results as reference. We calculated the number of pre-emptive isolation days avoided and incremental costs of adding RDT. RESULTS: A total of 163 patients at risk for MRSA carriage were screened and MRSA prevalence was 3.1% (n=5). Duration of isolation was 27.6 and 21.4 hours with IDI and GeneXpert, respectively, and would have been 96.0 hours when based on conventional cultures. The negative predictive value was 100% for both tests. Numbers of isolation days were reduced by 44.3% with PCR-based screening at the additional costs of euro327.84 (IDI) and euro252.14 (GeneXpert) per patient screened. Costs per isolation day avoided were euro136.04 (IDI) and euro121.76 (GeneXpert). CONCLUSIONS: In a low endemic setting for MRSA, RDT safely

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reduced the number of unnecessary isolation days on ICUs by 44%, at the costs of euro121.76 to euro136.04 per isolation day avoided.

Free full text available from PubMed
PMID: 22314204

5.3 Mass Spectrometry Applications for Infectious Disease

A) Are We Ready for Novel Detection Methods to Treat Respiratory Pathogens in Hospital-Acquired Pneumonia?
Endimiani A, Hujer KM, Hujer AM, et al. Are we ready for novel detection methods to treat respiratory pathogens in hospital-acquired pneumonia? *Clin Infect Dis*. 2011 May;52 Suppl 4:S373-83.

Summary: Hospital-acquired pneumonia represents one of the most difficult treatment challenges in infectious diseases. Many studies suggest that the timely administration of appropriate, pathogen-directed therapy can be lifesaving. Because results of culture and antimicrobial susceptibility testing can take 48 h or longer, physicians currently rely on clinical, epidemiological, and demographic factors to assist with the choice of empiric therapy for antibiotic-resistant pathogens. At present, a number of rapid molecular tests are being developed that identify pathogens and the presence of genetic determinants of antimicrobial resistance (eg, GeneXpert [Cepheid], ResPlex [Qiagen], FilmArray [Idaho Technologies], and Microarray [Check-Points]). In this review, the potential impact that molecular diagnostics has to identify and characterize pathogens that cause hospital-acquired bacterial pneumonia at an early stage is examined. In addition, a perspective on a novel technology, polymerase chain reaction followed by electrospray ionization mass spectrometry, is presented, and its prospective use in the diagnosis of pneumonia is also discussed. The complexities of the pulmonary microbiome represent a novel challenge to clinicians, but many questions still remain even as these technologies improve.

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PMID: 21460299

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B) RT-PCR/Electrospray Ionization Mass Spectrometry Approach in Detection and Characterization of Influenza Viruses

Deyde VM, Sampath R, Gubareva LV. RT-PCR/electrospray ionization mass spectrometry approach in detection and characterization of influenza viruses. *Expert Rev Mol Diagn*. 2011 Jan;11(1):41-52.

Summary: Reverse-transcription PCR (RT-PCR) coupled with electrospray ionization mass spectrometry (ESI-MS) is a high-throughput nucleic acid-based technology that relies on the accurate measurement of the molecular weight of PCR amplicons that can be used to deduce the base counts (number of As, Gs, Cs and Ts) of DNA. These amplicons represent highly variable regions with information-rich sequences, which are flanked by broad-range primers designed based on highly conserved loci. This technology was first introduced in 2005 for microbial identification and subtyping, and was later applied to influenza virus detection and identification. The influenza RT-PCR/ESI-MS assay allows analysis of approximately 300 samples per 24 h, and aids in the characterization of influenza viruses based on their 'core' gene signatures. Notably, this assay was used to identify one of the first cases of the 2009 H1N1 pandemic viruses. One of the main advantages of the RT-PCR/ESI-MS technology is its universality and adaptability for pathogen characterization. Efforts are being made to customize the currently used influenza surveillance assay for use in the diagnosis of the H1N1 pandemic virus. In this article, we provide a summary of known applications of the RT-PCR/ESI-MS assay in the field of influenza.

Full text available from *Expert Review of Molecular Diagnostics* (USD 60.00 for 24 hours access)
PMID: 21171920

C) MALDI Imaging Mass Spectrometry--Painting Molecular Pictures

Schwamborn K, Caprioli RM. MALDI imaging mass spectrometry--painting molecular pictures. *Mol Oncol*. 2010 Dec;4(6):529-38.

Summary: MALDI Imaging Mass Spectrometry is a molecular analytical technology capable of simultaneously measuring multiple

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analytes directly from intact tissue sections. Histological features within the sample can be correlated with molecular species without the need for target-specific reagents such as antibodies. Several studies have demonstrated the strength of the technology for uncovering new markers that correlate with disease severity as well as prognosis and therapeutic response. This review describes technological aspects of imaging mass spectrometry together with applications in cancer research.

Free full text available from *Molecular Oncology*
PMID: 20965799

D) New Technology for Rapid Molecular Diagnosis of Bloodstream Infections

Ecker DJ, Sampath R, Li H, et al. New technology for rapid molecular diagnosis of bloodstream infections. *Expert Rev Mol Diagn*. 2010 May;10(4):399-415.

Summary: Technologies for the correct and timely diagnosis of bloodstream infections are urgently needed. Molecular diagnostic methods have yet to have a major impact on the diagnosis of bloodstream infections; however, new methods are being developed that are beginning to address key issues. In this article, we discuss the key needs and objectives of molecular diagnostics for bloodstream infections and review some of the currently available methods and how these techniques meet key needs. We then focus on a new method that combines nucleic acid amplification with mass spectrometry in a novel approach to molecular diagnosis of bloodstream infections.

Free full text available from *Expert Review of Molecular Diagnostics*
PMID: 20465496

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Section 6 Pharmacogenomics

A) Predicting the Cost and Pace of Pharmacogenomic Advances: An Evidence-based Study

Arnaout R, Buck TP, Roulette P, Sukhatme VP. Predicting the cost and pace of pharmacogenomic advances: an evidence-based study. Clin Chem. 2013;59:649-57.

Summary: Adverse outcomes associated with prescription drug use are common and costly. Many adverse outcomes can be avoided through pharmacogenomics: choosing and dosing of existing drugs according to a person's genomic variants. Finding and validating associations between outcomes and genomic variants and developing guidelines for avoiding drug-related adverse outcomes will require further research; however, no data-driven estimates yet exist for the time or money required for completing this research. METHODS: We identified examples of associations between adverse outcomes and genomic variants. We used these examples to estimate the time and money required to identify and confirm other associations, including the cost of failures, and to develop and validate pharmacogenomic dosing guidelines for them. We built a Monte Carlo model to estimate the time and financial costs required to cut the overall rate of drug-related adverse outcomes by meaningful amounts. We analyzed the model's predictions for a broad range of assumptions. RESULTS AND CONCLUSIONS: Our model projected that the development of guidelines capable of cutting overall drug-related adverse outcomes by 25%-50% with current approaches will require investment of single-digit billions of dollars and take 20 years. The model forecasts a pump-priming phase of 5-7 years, which would require expenditures of hundreds of millions of dollars, with little apparent return on investment. The single most important parameter was the extent to which genomic variants cause adverse outcomes. The size of the labor force was not a limiting factor. A "50 000 Pharmacogenomes Project" could speed progress. Our approach provides a template for other areas of genomic research.

Full text available from *Clinical Chemistry* (USD 15.00 for 24 hour

access)
PMID: 23230323

B) Knowledge and Attitudes Concerning Pharmacogenomics Among Healthcare Professionals

Dodson C. Knowledge and attitudes concerning pharmacogenomics among healthcare professionals. *Personalized Medicine.* 2011 July 2011; 8(4): 421-428.

Summary: Pharmacogenomics has become an area of great potential in the medical community. Therefore, the assessment of the knowledge and attitudes among healthcare professionals is essential. The purpose of this systematic literature review is to explore the knowledge and attitudes of healthcare professionals regarding pharmacogenetic testing with a specific emphasis in oncology. A total of 12 articles were found and reviewed. A majority of the articles reported only on the attitudes of healthcare professionals. Four of the articles reported on both knowledge and attitudes of healthcare professionals concerning pharmacogenetic testing, and one article reported only on the knowledge level of healthcare professionals. This systematic literature review revealed that healthcare professionals generally perceive themselves to have limited knowledge regarding pharmacogenetic testing. In addition, these articles highlighted the overwhelming ethical concerns surrounding pharmacogenomics. However, these articles also revealed that healthcare professionals believed that there were also many advantages regarding the utilization of pharmacogenomics.

Full text available from *Personalized Medicine*

C) Genotype-based Dosing Algorithms for Warfarin Therapy: Data Review and Recommendations

Johnson EG, Horne BD, Carlquist JF, Anderson JL. Genotype-based dosing algorithms for warfarin therapy: data review and recommendations. *Mol Diagn Ther.* 2011 Oct 1; 15(5): 255-264.

Summary: Warfarin, the most common oral anticoagulant, is the ideal candidate for pharmacogenetic dosing and gene-based 'individualization' of care. A plethora of studies have shown that stable

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dose requirements can be predicted using sequence variants in the CYP2C9 and VKORC1 genes in both sexes and in different races. Multiple clinical trials of pharmacogenetic warfarin dosing have been conducted with various methods, including several randomized trials that have been completed. These studies have reported varying degrees of success and some have been met with substantial skepticism. Other much larger randomized trials are ongoing. This paper reviews and synthesizes the various clinical trials that have been published and touches on the potential that the ongoing trials offer. The emergence of new oral anticoagulants also raises the question of the relevance of pharmacogenetic warfarin dosing for the future. The cost of genotype-guided dosing is substantial, and none of the studies to date have shown a cost-benefit of using pharmacogenetic warfarin dosing in clinical practice. Although pharmacogenetics-guided warfarin dosing has been discussed for many years, the currently available data regarding this genetically individualized dosing suggest that pharmacogenetics remains unproven for use in clinical warfarin prescription.

Full text available from *Molecular Diagnosis & Therapy*
PMID: 22047153

D) Genetics of IL28B and HCV--Response to Infection and Treatment

Hayes CN, Imamura M, Aikata H, Chayama K. Genetics of IL28B and HCV--response to infection and treatment. *Nat Rev Gastroenterol Hepatol.* 2012 Jul; 9(7): 406-417.

Summary: The IL28B locus attracted the attention of HCV researchers after a series of genome-wide association studies independently identified a strong association between common IL28B polymorphisms and the outcome of PEG-IFN-alpha plus ribavirin combination therapy in patients chronically infected with HCV genotype 1. This association was subsequently replicated for other HCV genotypes and has been linked to spontaneous eradication of HCV, development of steatosis and biochemical changes (such as altered levels of gamma-glutamyl transpeptidase and LDL). Despite the introduction of direct-acting antiviral drugs, IL28B genetics are likely to play a part in patient selection and treatment decisions-

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moving towards a personalized approach to therapy. In HCV-infected patients with the so-called favourable IL28B genotype (rs12979860 CC; associated with better treatment response), hepatic expression levels of IL28B and interferon-stimulated genes seem to be reduced at baseline, but are induced more strongly after IFN-alpha administration, perhaps resulting in more effective elimination of the virus. Clarification of the mechanisms underlying these biological phenomena will lead to improved understanding of the antiviral effects of IFN-lambda and, ideally, to the development of better therapies against HCV infection. This Review summarizes current understanding of the role of IL28B in HCV infection and response to therapy.

Full text available from *Nature Reviews Gastroenterology and Hepatology* (USD 32.00)
PMID: 22641049
NOTE: Also cited in Section 5.2.1.5

E) Prediction of Warfarin Dose: Why, When and How?
Eriksson N, Wadelius M. Prediction of warfarin dose: why, when and how? *Pharmacogenomics.* 2012 Mar; 13(4): 429-440.

Summary: Prediction models are the key to individualized drug therapy. Warfarin is a typical example of where pharmacogenetics could help the individual patient by modeling the dose, based on clinical factors and genetic variation in CYP2C9 and VKORC1. Clinical studies aiming to show whether pharmacogenetic warfarin dose predictions are superior to conventional initiation of warfarin are now underway. This review provides a broad view over the field of warfarin pharmacogenetics from basic knowledge about the drug, how it is monitored, factors affecting dose requirement, prediction models in general and different types of prediction models for warfarin dosing.

Free full text available from *Pharmacogenomics*
PMID: 22379999

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F) Genetic Determinants of Response to Clopidogrel and Cardiovascular Events

Simon T, Verstuyft C, Mary-Krause M, et al. Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med.* 2009 Jan 22; 360(4): 363-375.

Summary: Pharmacogenetic determinants of the response of patients to clopidogrel contribute to variability in the biologic antiplatelet activity of the drug. The effect of these determinants on clinical outcomes after an acute myocardial infarction is unknown. METHODS: We consecutively enrolled 2208 patients presenting with an acute myocardial infarction in a nationwide French registry and receiving clopidogrel therapy. We then assessed the relation of allelic variants of genes modulating clopidogrel absorption (ABCB1), metabolic activation (CYP3A5 and CYP2C19), and biologic activity (P2RY12 and ITGB3) to the risk of death from any cause, nonfatal stroke, or myocardial infarction during 1 year of follow-up. RESULTS: Death occurred in 225 patients, and nonfatal myocardial infarction or stroke in 94 patients, during the follow-up period. None of the selected single-nucleotide polymorphisms (SNPs) in CYP3A5, P2RY12, or ITGB3 were associated with a risk of an adverse outcome. Patients with two variant alleles of ABCB1 (TT at nucleotide 3435) had a higher rate of cardiovascular events at 1 year than those with the ABCB1 wild-type genotype (CC at nucleotide 3435) (15.5% vs. 10.7%; adjusted hazard ratio, 1.72; 95% confidence interval [CI], 1.20 to 2.47). Patients carrying any two CYP2C19 loss-of-function alleles (*2, *3, *4, or *5), had a higher event rate than patients with none (21.5% vs. 13.3%; adjusted hazard ratio, 1.98; 95% CI, 1.10 to 3.58). Among the 1535 patients who underwent percutaneous coronary intervention during hospitalization, the rate of cardiovascular events among patients with two CYP2C19 loss-of-function alleles was 3.58 times the rate among those with none (95% CI, 1.71 to 7.51). CONCLUSIONS: Among patients with an acute myocardial infarction who were receiving clopidogrel, those carrying CYP2C19 loss-of-function alleles had a higher rate of subsequent cardiovascular events than those who were not. This effect was particularly marked among the patients undergoing percutaneous coronary intervention. (ClinicalTrials.gov number, NCT00673036.)

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Free full text available from *New England Journal of Medicine*
PMID: 19106083

G) Variants in Tamoxifen Metabolizing Genes: A Case-Control Study of Contralateral Breast Cancer Risk in the WECARE Study
Brooks JD, Teraoka SN, Malone KE, et al. Variants in tamoxifen metabolizing genes: a case-control study of contralateral breast cancer risk in the WECARE study. *Int J Mol Epidemiol Genet.* 2013 4(1): 35-48.

Summary: Tamoxifen has been shown to greatly reduce risk of recurrence and contralateral breast cancer (CBC). Still, second primary contralateral breast cancer is the most common malignancy to follow a first primary breast cancer. Genetic variants in CYP2D6 and other drug-metabolizing enzymes that alter the metabolism of tamoxifen may be associated with CBC risk in women who receive the drug. This is the first study to investigate the impact of this variation on risk of CBC in women who receive tamoxifen. From the population-based Women's Environment Cancer and Radiation Epidemiology (WECARE) Study, we included 624 Caucasian women with CBC (cases) and 1,199 women with unilateral breast cancer (controls) with complete information on tumor characteristics and treatment. Conditional logistic regression was used to assess the risk of CBC associated with 112 single nucleotide polymorphisms (SNPs) in 8 genes involved in the metabolism of tamoxifen among tamoxifen users and non-users. After adjustment for multiple testing, no significant association was observed between any of the genotyped variants and CBC risk in either tamoxifen users or non-users. These results suggest that when using a tagSNP approach, common variants in selected genes involved in the metabolism of tamoxifen are not associated with risk of CBC among women treated with the drug.

Free full text available from PubMed
PMID: 23565321

H) Genomics and Drug Response
Wang L, McLeod HL, Weinshilbourn RM. Genomics and drug response. N Engl J Med. 2011 Mar:364:1144-53.

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Summary: Pharmacogenomics is the study of the role of inherited and acquired genetic variation in drug response.1 Clinically relevant pharmacogenetic examples, mainly involving drug metabolism, have been known for decades, but recently, the field of pharmacogenetics has evolved into “pharmacogenomics,” involving a shift from a focus on individual candidate genes to genomewide association studies. Such studies are based on a rapid scan of markers across the genome of persons affected by a particular disorder or drug-response phenotype and persons who are not affected, with tests for association that compare genetic variation in a case–control setting. An example is provided in this issue of the *Journal:* McCormack and colleagues, testing for genomewide association, identified an HLA allele that is associated with hypersensitivity reactions to the anticonvulsant and mood-stabilizing drug carbamazepine in persons of European descent. Pharmacogenomics facilitates the identification of biomarkers that can help physicians optimize drug selection, dose, and treatment duration and avert adverse drug reactions. In addition, pharmacogenomics can provide new insights into mechanisms of drug action and as a result can contribute to the development of new therapeutic agents.

Free full text available from PubMed
PMID: 21428770

I) Update on CYP2D6 and its Impact on Tamoxifen Therapy

Goetz MP. Update on CYP2D6 and its impact on tamoxifen therapy. *Clin Adv Hematol Oncol*. 2010 Aug 8(8):536-538.

Abstract not available.

Full text available from *Clinical Advances of Hematology & Oncology* (registration required)
PMID: 20966889

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J) Exemestane for Breast-cancer Prevention in Postmenopausal Women

Goss PE, Ingle JN, Ales-Martinez JE, et al. Exemestane fror Breast-Cancer Prevention in Postmenopausal Women. NEJM. 2011 Jun:364(25):2381-2391.

Summary: Tamoxifen and raloxifene have limited patient acceptance for primary prevention of breast cancer. Aromatase inhibitors prevent more contralateral breast cancers and cause fewer side effects than tamoxifen in patients with early-stage breast cancer. METHODS: In a randomized, placebo-controlled, double-blind trial of exemestane designed to detect a 65% relative reduction in invasive breast cancer, eligible postmenopausal women 35 years of age or older had at least one of the following risk factors: 60 years of age or older; Gail 5-year risk score greater than 1.66% (chances in 100 of invasive breast cancer developing within 5 years); prior atypical ductal or lobular hyperplasia or lobular carcinoma in situ; or ductal carcinoma in situ with mastectomy. Toxic effects and health-related and menopause-specific qualities of life were measured. RESULTS: A total of 4560 women for whom the median age was 62.5 years and the median Gail risk score was 2.3% were randomly assigned to either exemestane or placebo. At a median follow-up of 35 months, 11 invasive breast cancers were detected in those given exemestane and in 32 of those given placebo, with a 65% relative reduction in the annual incidence of invasive breast cancer (0.19% vs. 0.55%; hazard ratio, 0.35; 95% confidence interval [CI], 0.18 to 0.70; P=0.002). The annual incidence of invasive plus noninvasive (ductal carcinoma in situ) breast cancers was 0.35% on exemestane and 0.77% on placebo (hazard ratio, 0.47; 95% CI, 0.27 to 0.79; P=0.004). Adverse events occurred in 88% of the exemestane group and 85% of the placebo group (P=0.003), with no significant differences between the two groups in terms of skeletal fractures, cardiovascular events, other cancers, or treatment-related deaths. Minimal quality-of-life differences were observed. CONCLUSIONS: Exemestane significantly reduced invasive breast cancers in postmenopausal women who were at moderately increased risk for breast cancer. During a median follow-up period of 3 years, exemestane was associated with no serious toxic effects and only minimal changes in health-related quality of life. (Funded by

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Pfizer and others; NCIC CTG MAP.3 ClinicalTrials.gov number, NCT00083174.).

Free full text available from *New England Journal of Medicine*
PMID: 21639806

K) Pharmacogenomics and the Future of Toxicology Testing
Agrawal YP, Rennert H. Pharmacogenomics and the future of toxicology testing. *Clin Lab Med.* 2012 Sep;32(3):509-23.

Summary: Pharmacogenomics is a useful tool in clinical toxicology for characterizing many gene polymorphisms associated with different pharmacokinetics or pharmacodynamics of exogenously administered drugs. These genetic variants may determine ranges of variation in such fundamental aspects as drug-metabolizing enzymes, drug transporters, drug receptors, or targets of drug action. Toxicologically significant drugs for which the FDA has required the manufacturer to identify relevant pharmacogenomics markers on the label include carisoprodol, citalopram, codeine, and risperidone. For personalized medicine, combining pharmacogenomics testing with therapeutic drug monitoring may allow the identification of individuals who need lower or higher doses, or even a different drug.

Full text available from *Toxicology Testing* (USD 31.50)
PMID: 22939305

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Section 7 CAP Resources: Guidelines, Accreditation, and Proficiency Testing

7.1 CAP Guidelines

The College of American Pathologists developed the Pathology and Laboratory Quality Center, or "the CAP Center," as a forum to author and maintain evidence-based guidelines and consensus statements. For more information about the CAP Center and its process, please visit the CAP Guidelines website.

The CAP Center has several guidelines published and in development in the area of molecular testing. Please follow the links for more information about each of these Center guidelines and other related resources.

Published:

- ASCO/CAP ER/PgR Guideline and Resources
- ASCO/CAP HER2 Testing Guidelines and Resources (Update)
- CAP/ASCCP Lower Anogenital Squamous Terminology (LAST) for HPV-Associated Lesions: Consensus Recommendations and Resources
- CAP/IASLC/AMP Molecular Testing Guidelines for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors
- Principles of Analytic Validation of Immunohistochemical Assays

In Development:

- CAP/ASH Algorithm for Initial Work-up of Acute Leukemia
- ASCP/CAP/AMP Molecular Markers for the Evaluation of Colorectal Cancer
- Bone Marrow Synoptic Reporting for Hematologic Neoplasms
- HER2 Testing Guidelines for Gastric Cancer
- Detection of HPV in Head and Neck Squamous Cell Carcinomas

For the latest status updates, please check the CAP Center Upcoming Guidelines page.

A) CAP/NSH Histotechnology Committee Guidelines for Pre-Microscopic Examination in Surgical Pathology
CAP/NSH Histotechnology Committee. CAP/NSH Histotechnology Committee Guidelines for Pre-Microscopic Examination in Surgical Pathology. 2013.

Summary: In spite of the abundant guidelines and recommendations published for specimen handling and testing in a clinical pathology laboratory, relatively little literature is available for guidance of specimen handling in a surgical pathology laboratory. This document does not relate to cytologic or clinical pathology samples.

The following comprehensive table is intended to serve as a general guideline for proper specimen handling from the time it is taken from the patient to the time a completed slide of the specimen is given to a pathologist for interpretation. This document was created by members of the CAP/NSH Histotechnology Committee and is intended to serve as a guideline and NOT absolute recommendations for specimen handling. Each laboratory is advised to use these guidelines as a starting point and modify certain parameters to fit local institutional needs, as appropriate. Whenever appropriate, regulatory references for certain guidelines are provided in the table.

It is the intent of the CAP/NSH Histotechnology Committee to update this document every 2 years or so and have the updated version of the document available on the College of American Pathologists (CAP) and National Society for Histotechnology (NSH) websites.

Access full table

7.2 CAP Cancer Biomarker Reporting Templates

In an effort to improve consistency and completeness in reporting results of cancer biomarker testing, the College of American Pathologists (CAP) has produced standardized templates for the reporting of cancer

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biomarker testing. These templates were developed to respond to pathologist user feedback about timing of reporting and structural consistency of ancillary studies data elements in the CAP Cancer Protocols and to provide educational materials to help pathologists better understand emerging biomarkers. The development of these templates has been a collaborative effort between CAP, AMP, ASCO, CDC, AJCC and many other participating organizations.

Published as of June 2014:

- Introducing Reporting Templates for Cancer Biomarkers
- Template for Reporting Results of Biomarker Testing of Specimens From Patients With Carcinoma of the Breast
- Template for Reporting Results of Biomarker Testing of Specimens From Patients With Carcinoma of the Colon and Rectum
- Template for Reporting Results of Biomarker Testing of Specimens From Patients With Non-Small Cell Carcinoma of the Lung
- Template for Reporting Results of HER2 (ERBB2) Biomarker Testing of Specimens from Patients with Adenocarcinoma of the Stomach or Esophagogastric Junction

Access:

All current CAP Cancer Biomarker Templates are publicly available in Microsoft Word and PDF format. They can be found on the www.cap.org website by navigating to the Reference Resources and Publications tab, or by navigating directly to www.cap.org/cancerprotocols.

In Development:

- Template for Reporting Results of Biomarker Testing of Specimens From Patients With Tumors of the Central Nervous System
- Template for Reporting Results of Reporting Biomarker Testing of Specimens From Patients with Gastrointestinal Stromal Tumors
- Template for Reporting Results of Biomarker Testing of Specimens From Patients With Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma
- Template for Reporting Results of Monitoring Tests for Patients With Chronic Myelogenous Leukemia *(BCR-ABL1+)*

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- Template for Reporting Results of Biomarker Testing of Specimens From Patients With Diffuse Large B-Cell Lymphoma, Not Otherwise Specified
- Template for Reporting Results of Biomarker Testing for Myeloproliferative Neoplasms
- Template for Reporting Results of Biomarker Testing of Specimens From Patients With Carcinoma of the Endometrium
- Template for Reporting Results of Biomarker Testing of Specimens From Patients With Melanoma

For more information about the Cancer Biomarker Reporting Templates, please contact us at cprotoc@cap.org.

7.3 CAP Electronic Cancer Checklists (eCC)

For pathologists that would like to implement the CAP Cancer Biomarker Templates into their daily workflow within their laboratory information systems, the CAP offers an electronic version of these data entry forms through the CAP electronic Cancer Checklists (eCC).

Summary

The amount of information pathologists provide in their reports on cancer specimens has increased in recent years, due to the expansion of scientific knowledge about cancer and continued advances in health care, such as molecular diagnostics and personalized medicine. The CAP eCC, the electronic version the more than 80 case summaries currently within the CAP Cancer Protocols and Cancer Biomarker Templates, helps health care professionals manage information, as it offers a standardized way to report cancer data electronically.

The CAP eCC advances the management and interoperability of health information through its XML (Extensible Markup Language) format that can be integrated easily into existing pathology and cancer registry systems and also supports ever-evolving health IT platforms. They are, in part, coded with SNOMED Clinical Terms® (SNOMED CT®) that fosters multiple interdisciplinary providers to accurately communicate and share patient information within an electronic health record (EHR) system. Data

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can also be transmitted in real time, improving the timeliness, accuracy, and completeness of cancer reporting.
In addition, the CAP eCC, focusing directly on real-world medical content standards from physician-experts on the CAP's Cancer, Cancer Biomarker, and Pathology Electronic Reporting Committees, illustrates how technology directly supports meaningful use of patient data in the cancer-care setting.

Features

- XML format broadly adopted by health information technology (HIT) and endorsed by international standards organizations such as HL7, IHE, IHTSDO, etc
- Content includes elements from *AJCC Cancer Staging Manual* 7th Edition
- Regularly scheduled content releases and updates
- Mappings encoded with SNOMED CT, LOINC® (CS v2 capable)
- Easy integration into existing pathology and cancer registry systems
- Individual laboratory practice customization

Content Update

The CAP eCC releases contain elements from the AJCC Cancer Staging Manual 7th Edition. The CAP Cancer and Biomarker Reporting Committees update the content found within the CAP Cancer Protocols and Biomarker Templates online at www.cap.org/cancerprotocols. The CAP eCC releases are distributed to licensees with the addition of new or updated content, technical features, or mappings.

Licensing

The CAP eCC downloadable file set is available with a CAP eCC license. Vendor integration licensing options are also available. For more information, visit our website at www.cap.org/capecc, or contact us at 847-832-7700 or capecc@cap.org.

Goals

- Support and aid the pathologist in the diagnostic process
- Standardize the collection of pathology data to improve cancer reporting and research initiatives
- Advance the pathologist's role as chief diagnostician

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- Improve information sharing and support interoperability and data exchange to foster a more efficient cancer reporting process
- Promote worldwide adoption and utilization of structured pathology reporting

Usability

- Clinical practice
- Tumor Board
- Data mining
- Data analysis
- Epidemiological reporting
- Public health
- Quality improvement
- Cancer research
- Cancer surveillance
- Reimbursement
- Tissue banking

Content Update

The CAP eCC releases contain elements from the AJCC Cancer Staging Manual 7th Edition. The CAP Cancer Committee updates the content found within the CAP Cancer Protocols online at cap.org/cancerprotocols. The CAP eCC releases are distributed to licensees with the addition of new or updated content, technical features, or mappings.

For more information or for a demonstration of the CAP eCC, contact us at 847-832-7700 or capecc@cap.org.

7.4 CAP PT: Proficiency Testing

The College has many Surveys for molecular proficiency testing. Survey products covering cytogenetics, infectious disease, molecular oncology (solid tumors and hematologic), biochemical and molecular genetics can be found in the 2014 Surveys catalog.

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Sample Exchange Registry for Alternative Assessment
The Sample Exchange Registry is an Internet-based service designed to connect laboratories performing testing where no formal proficiency testing (PT) is available. This service now includes all clinical laboratory disciplines. Laboratories can participate in the registry service at any time. When at least three laboratories are identified as testing for the same analyte, the CAP will facilitate the sample exchange. Visit www.cap.org/sampleexchange for additional information and to register for an exchange.

For more information on the Sample Exchange Registry, please contact:
Patty Vasalos, Senior Technical Analyst, Surveys
College of American Pathologists
325 Waukegan Road
Northfield, IL 60093
1-800-323-4040 x 7584
exchangereg@cap.org

7.5 NGS Proficiency Testing Pilot

In line with CAP's mission to foster the highest standards in the practice of pathology, to advance the science of pathology, and to improve medical laboratory service, CAP's Next Generation Sequencing (NGS) Workgroup collaborated to enhance the CAP's Accreditation Programs Checklists for NGS and developed a methods-based proficiency test for NGS, demonstrating CAP's leadership in advancing standards of practice in genomic medicine.

- Labs that perform NGS for heritable diseases, pharmacogenetic markers, or somatic mutations can use this PT product to meet certain accreditation requirements
- Designed for laboratories both the wet bench and bioinformatics components
- For laboratories using gene panels, exome, and whole genome sequencing
- Customers will receive 10.0-µg extracted DNA specimen twice per year
- Customers will be able to test up to 200 gene variants in each sample

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If customers are interested in the proficiency testing information, it will be available in the 2015 Surveys catalog expected to be shipped September 1, 2014.

7.6 CAP LAP: Accreditation

The CAP Laboratory Accreditation Program is an internationally recognized program and the only one of its kind that utilizes teams of practicing laboratory professionals as inspectors. Designed to go well beyond regulatory compliance, the program helps laboratories achieve the highest standards of excellence to positively impact patient care. The program is based on rigorous accreditation standards that are translated into detailed and focused checklist requirements. The checklists, which provide a quality practice blueprint for laboratories to follow, are used by the inspection teams as a guide to assess the overall management and operation of the laboratory.

The Molecular Pathology Checklist (description below) is used, along with the All Common and Laboratory General Checklists, for inspection of laboratories performing testing using molecular methods. The checklists are available for download from eLAB Solutions or may be purchased as a set on the CAP website.

CAP continues to be actively engaged in updating the Molecular Pathology Checklist to reflect new technologies and applications. In the 2012 edition, the CAP introduced requirements on the clinical application of genomic analysis, more specifically next-generation sequencing (NGS), to prepare its laboratory customers for advancements in genomic testing. The 2013 edition was further expanded to contain new requirements for non-invasive screening of maternal plasma to identify fetal aneuploidy using next-generation sequencing.

Molecular Pathology Checklist description:
Testing that involves DNA/RNA probe hybridization or amplification constitutes molecular testing. The Molecular Pathology Checklist covers clinical molecular genetic testing in the areas of oncology, hematology,

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inherited disease, HLA typing, forensics and parentage applications. The Molecular Pathology Checklist is used to inspect a variety of methodologies, including polymerase chain reaction, arrays, fluorescence and non-florescence in situ hybridization, electrophoresis, Sanger sequencing, and next-generation sequencing.

The inspection of laboratories performing such molecular testing requires the Molecular Pathology checklist, with the following exceptions:

- The Cytogenetics or Anatomic Pathology checklist (as appropriate) may be used to inspect fluorescence in situ hybridization (FISH), when such testing is performed in the cytogenetics, cytopathology or anatomic pathology section.
- The Anatomic Pathology checklist may be used to inspect in situ hybridization (ISH), when ISH testing is performed in the anatomic pathology or cytopathology section.
- The Microbiology Checklist is used to inspect laboratories performing molecular testing for infectious diseases, including FDA-cleared/approved, modified FDA-cleared/approved, and laboratory-developed methods.
- This Histocompatibility Checklist is used to inspect HLA antigen typing performed using molecular methods for the purposes of transplantation.

Questions about the Molecular Pathology Checklist?
Gain insight from the experience of an inspector that performs numerous molecular laboratory inspections each year by listening to the 2014 CAP audio conference “Complying with Molecular Pathology Accreditation Requirements.” The discussion will focus on those standards from the Molecular Pathology Checklist that challenge laboratories.

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Section 8 CAP Molecular Testing Educational Resources

8.1 CAP Short Presentations on Emerging Concepts (SPECs)

Pathology Short Presentations on Emerging Concepts (SPECs)

Pathology SPECs are:

- Prewritten PowerPoint presentations on selected diseases where molecular tests play a key role in patient management.
- Focused on molecular tests that are actionable to patient care today.

Pathologists will find the SPECs an especially valuable resource as they facilitate discussion with Tumor Boards or other physician colleagues.

To view all the available SPECs, register by going to the CAP Member tab on cap.org.

- Prenatal Screening for Down Syndrome: Past, Present and Emerging
  Practices (NEW)
- HER2 Testing in Breast Cancer: 2013 ASCO/CAP HER2 Guideline Update (NEW)
- Emerging Concepts in the Diagnosis of Respiratory Viruses
- Emerging Concepts in Molecular Testing in Breast Cancer
- Emerging Concepts in the Workup of Colorectal Cancer
- Emerging Concepts in Therapeutic Guidance for Metastatic Melanoma
- Emerging Concepts in the Diagnosis and Workup of Thyroid Cancer
- Emerging Concepts in Colorectal Cancer Hereditary Non-Polyposis Cancer (Lynch Syndrome)

- Emerging Concepts in the Workup of Polycythemia and Thrombocythemia: *JAK2*

8.2 CAP Webinars

CAP has webinars focused on informing pathologists on key genomic and molecular medicine topics. Since 2009, the "Hot Topics in Pathology" webinar series presents webinars on 1) oncology molecular topics 2) getting started in molecular/next steps in molecular topics and 3) next-generation sequencing topics. These webinars are complimentary and do not have CME.

New in 2014, “Practical Genomics for the Practicing Pathologist” will present specific genomic skills that are needed for pathologists. These webinars will provide 1 hour CME and have a small fee.

To view new or archived webinars, go to www.cap.org/webinars.

Webinar Title	Date/Time (US Central Time)
Genomic Testing: What is it Good For?	July 17, 2014 @ 12 pm
Common Cancer Genes Used by NGS Pathologists Early Adopters Panels	August 27, 2014 @ 1 pm
The Critical First Steps: Specimen Acquisition and Handling for Cancer Genomics	September 24, 2014 @ 11 am
Economics 101: In House Versus Reference Testing - Criteria to Consider for Molecular Tests	October 22, 2014 @ 11 am
Garbage in, Garbage out: How Every Pathologist Can Ensure Accurate Genomic Oncologic Testing	November 20, 2014 @ 12 pm
Cancer Genomics: Selecting the Right Test at the Right Time	December 11, 2014 @ 12 pm

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8.2.1 2014 Hot Topics in Pathology Webinars

DATE	TOPIC	SPEAKER(s)
Oct 22 11am CT	Economics 101: In House Versus Reference Testing - Criteria to Consider for Molecular Tests	Jordan S. Laser, MD, FCAP

8.2.2 Practical Genomics for the Practicing Pathologist Webinar Series

- The College of American Pathologists is pleased to release this new webinar series on critical genomic testing knowledge and skills.
- Each hour-long webinar highlights a critical genomics skill that practicing pathologists will find relevant, practical, and timely.
- Each webinar carries 1 *AMA PRA Category 1 Credit*™. And because the series supports advancing the specialty as well as CAP's Genomics Strategy, each is available to members for just $10.

Practical Genomics for the Practicing Pathologist Webinar Series	Date/Time (US Central Time)
Genomic Testing: What is it Good For?	July 17, 2014 @ 12 pm
The Critical First Steps: Specimen Acquisition and Handling for Cancer Genomics	September 24, 2014 @ 11 am
Garbage in, Garbage out: How Every Pathologist Can Ensure Accurate Genomic Oncologic Testing	November 20, 2014 @ 12 pm
Cancer Genomics: Selecting the Right Test at the Right Time	December 11, 2014 @ 12 pm

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8.2.3 Archived Webinars – Getting Started in Molecular Pathology

TOPIC	SPEAKER(s)
Cancer Test Reporting for Breast, Colorectal and Lung Biomarkers: New CAP Templates to Help You and Your Practice. Presented May 29, 2014. Archived webinar available for free; presentation slides available	Pat Fitzgibbons, MD, FCAP & George Birdsong, MD, FCAP
IHC Assays – New Evidence-Based Guideline for Analytic Validation. Presented April 1, 2014. Archived webinar available for free; presentation slides available	Jeffrey Goldsmith, MD, FCAP
Prenatal Screening for Down Syndrome: Past, Present and Emerging Practices Testing Maternal Plasma DNA for Down Syndrome. Presented March 20, 2014. Archived webinar available for free; presentation slides available	Glenn Palomaki, PhD
Viral Respiratory Tract Infections: Detection Now and in the Future. Presented February 27, 2014. Archived webinar available for free; presentation slides available	Frederick L Kiechle MD, PhD, FCAP
Practical Issues in Surgical Pathology that Enhance Ancillary Molecular Testing for Cancer. Presented January 28, 2014. Archived webinar available for free; presentation slides available	John D Pfeifer, MD, PhD, FCAP
Transforming the Diagnostic Evaluation of Inherited Disorders with Next-Generation Sequencing. Presented April 23, 2013. Archived webinar available for free; presentation	Karl V Voelkerding, MD, FCAP

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slides available	
Making the Case for Pharmacogenomics Testing: Integration into a Health Care System. Presented February 26, 2013. Archived webinar available for free; presentation slides available	Michael Laposata, MD, PhD, FCAP
The Business Argument for Cancer Genomic Testing. Presented December 7, 2012. Archived webinar available for free; presentation slides available	James Crawford, MD, PhD, FCAP, John Pfeifer, MD, PhD, FCAP, Lynn Bry, MD, PhD, FCAP
The Legal Status of Patents on Genomic Lab Tests. Presented on October 10, 2012. Archived webinar available for free; presentation slides available	Jack Bierig, JD

8.2.3 Archived Webinars – Organ Based Pathology

TOPIC	SPEAKER(s)
Applying the CAP-ASCCP Lower Anogenital Squamous Terminology Project (LAST) Principles in Clinical Practice: Case examples illustrating biomarker usage. Presented November 6, 2013. Archived webinar available for free; presentation slides available	Brigitte M. Ronnett, MD
HER2 Testing Revision. Presented December 3, 2013. Archived webinar available for free; presentation slides available	David G. Hicks, MD, FCAP and Stephen J. Sarewitz, MD, FCAP
Molecular Markers in Breast Cancer. Presented March 20, 2013. Archived webinar available for free; presentation slides available	David G. Hicks, MD, FCAP

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Biomarkers in HPV-associated Lower Anogenital Squamous Lesions from the CAP-ASCCP Lower Anogenital Squamous Terminology Project. Presented September 27, 2012. Archived webinar available for free; presentation slides available	Mark Stoler, MD, FCAP

8.3 CAP Annual Conference

CAP 14 – THE Pathologists' Meeting™

September 7-10, 2014
Hyatt Regency Chicago
Chicago, Illinois

The CAP is offering the following courses/presentations covering molecular testing topics at CAP '14.

Course Code	Title	Presenter(s)
H1381	Gastrointestinal Pathology: Bridging the Gap Between Molecular and Practical	Robert E. Petras, MD, FCAP
V1308	Diagnosis of Urinary Tract Lesions Using Integrated Histological, Cytological, and Molecular FISH Approaches	Longwen Chen, MD, PhD, FCAP, Ming Zhou, MD, PhD, FCAP
S1307	Practical Integration of Clinical, Electrophoretic, and Molecular Features of Hemoglobin Disorders	James D. Hoyer, MD Jennifer L. Oliveira, MD
S1240	Molecular Microbiology in the Community-Based Practice	Cindy B. McCloskey, MD, FCAP
S1289	Essentials of CPT Coding: Cytopathology, Surgical Pathology, and Molecular Pathology	Susan E. Spires, MD, FCAP Mark S. Synovec, MD, FCAP

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8.4 CAP Education

CAP's online learning portal has online resources for education by the experts.

CAP SAMs (Self-Assessment Modules)
Consistent with the high quality you expect from the College, CAP SAMs are more comprehensive than simple "memory tests." CAP SAM topics are identified as critical areas of importance to pathologists, and they include a strong focus on both the interpretation of concepts and their direct application to patient care.

CAP SAMs include both educational content and a multiple-choice post-test. Immediate feedback is provided after each question, providing additional educational material on both correct and incorrect responses. Participants who earn a passing score may apply their earned credit(s) to the American Board of Pathology's Maintenance of Certification SAM requirements.

Title/Description	CME/SAM
Basic Concepts in Genetics **This course is a review of genetic principles and terminology which serve as the basis for understanding pharmacogenomics and pharmacogenetics clinical testing.**	***2***
Pharmacokinetics and Pharmacodynamics Underlying Pharmacogenomic Testing **This course will provide opportunities to review the pharmacokinetic and pharmacodynamic concepts (eg, volume of distribution, elimination rate, half-life, clearance, and area under the curve) needed to understand the molecular study (pharmacogenomic testing) of genetic factors that determine drug efficacy and toxicity.**	***2***

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Methods in Pharmacogenomics Testing and Clinical Applications **This course presents the techniques and technologies in the context of their clinical applications in drug metabolism and cancer and provides information on how to ensure quality molecular test results.**	***2.5***
Pharmacogenomics in Medical Practice **This course applies the concepts and principles of molecular situations.**	***3.5***

Archives Applied

The Archives Applied CME/SAM program is designed for pathologists and includes educational content and a multiple-choice post-test based on select articles from the *Archives of Pathology & Laboratory Medicine* journal. Immediate feedback is provided after each question, and a post-test score is provided upon completion. Participants who earn a passing score on the post-test may apply their earned credit to the American Board of Pathology's (ABP) Maintenance of Certification (MOC) SAM requirements. All participants who complete the activity can claim CME credit.

Title/Description	CME/SAM
Archives Applied: Molecular Pathology of Breast Cancer This self assessment module (SAM) is based on an article that appeared in the May 2011 issue of Archives of Pathology & Laboratory Medicine about molecular pathology of breast cancer. It was developed by an expert in the field, Dr. Aaron M. Gruver.	***1.0***
Archives Applied - Response to Targeted Agents for Kidney Cancer (SAM eligible) This SAM is designed for pathologists to heighten their awareness of predictors of response to targeted therapy in renal cell carcinoma. It was developed by experts in the field, Ximing J. Yang and Sanjiv V. Prabhu.	***1.0***

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Archives Applied - Analytical Validation of a Sequencing Assay (SAM eligible) This SAM is designed for pathologists to heighten their awareness of the design and analytical validation of clinical DNA sequencing assays. It was developed by an expert in the field, Elaine Lyon.	*1.0*
Archives Applied: Echinoderm Microtubule-Associated Proteinlike 4-Anaplastic Lymphoma Kinase (EML4-ALK) Mutational Analysis This self assessment module (SAM) is based on an article that appeared in the January 2011 issue of Archives of Pathology & Laboratory Medicine about the detection of EML4-ALK fusion-positive non-small cell lung carcinoma. It was developed by an expert in the field, Lester J. Layfield.	*1.0*
Archives Applied - CXCR4 Expression in Chondrosarcoma (SAM eligible) This self assessment module (SAM) is designed for pathologists to heighten their awareness of CXCR4 expression in chondrosarcoma of bone. It was developed by an expert in the field, Dr. Gene P. Siegal.	*1.0*

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Molecular Pathology eLearning

Developed by members of the CAP Molecular Oncology committee, these activities have a unique course design:

- Models critical molecular oncology competencies – knowledge, skills and behaviors – and reinforces key messages through knowledge checks, discussions and self-reflection.
- Desired performance behaviors are modeled by characters in the story, which demonstrate a highly diversified view of patient/pathologist interactions with respect to molecular oncology.

Title/Description	CME/SAM
BRAF Mutations in Thyroid Cases The intent of this course is to help you build capability in Molecular Oncology to immediately improve the quality of patient care. It will provide you with information and techniques to evaluate your knowledge and skills in various aspects of molecular oncology and integrate this information for the benefit of patients.	*.5*
Classification and Clinical Management of Myeloproliferative Neoplasms with Eosinophilia (SAM eligible) This course uses a case-based approach to illustrate the utilization of the updated classification scheme, including new terminology, relevant clinical findings, peripheral blood smear morphology, bone marrow aspirate smears and histology, and laboratory/molecular testing.	*1.25*
BPF Testing Self Study 2014 Technical Update (SAM eligible) In October 2013, recommendations in the 2007 ASCO-CAP Guideline for HER2 Testing have been updated with detailed recommendations for how to test for HER2 overexpression, interpret the results, and recommend HER2-targeted therapies.	*3.75*

CAP Advanced Practical Pathology Programs

CAP Advanced Practical Pathology Programs (AP3) offer pathologists the opportunity to develop, demonstrate and be recognized for knowledge and skills in areas not currently addressed by the American Board of Pathology (ABP).

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To receive a CAP AP3 Certificate of Recognition, applicants need to meet four key requirements:

- Board Certification in anatomic and/or clinical pathology from the ABP, the American Osteopathic Board of Pathology (AOBP), or the Royal College of Physicians and Surgeons of Canada (RCPS).
- Completion of Continuing Medical Education in the program's area of special expertise. This will vary with each AP3.
- Successful completion of a cognitive assessment that objectively measures practical knowledge in the program area of expertise.
- Successful completion of one or more practical assessments that measure performance in the program area.
-

Course Code	Title/Description
BPFT AP3	**Breast Predictive Factors Testing Advanced Practical Pathology Program** The CAP's BPFT AP3 is designed to facilitate appropriate treatment of breast cancer patients by helping to ensure accurate evaluation and interpretation of breast cancer predictive factors.
MBP AP3	**Multidisciplinary Breast Pathology Advanced Practical Pathology Program** The MBP AP3 provides pathologists with the focused training, skills and education to effectively deliver their critical expertise across increasingly integrated, multidisciplinary breast cancer care teams.

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Section 9 Other Industry Resources and Conferences

Following is a sampling of resources for molecular testing available outside of the CAP. The listings here are not necessarily endorsed by the CAP.

A) **Michigan State University Molecular Laboratory Diagnostics Program**

The Michigan State University (MSU) Biomedical Laboratory Diagnostics (BLD) program offers an online post-baccalaureate certificate program in Molecular Laboratory Diagnostics. Upon successful completion of this three-course program, participants will receive a certificate of completion; however, individual course enrollment is possible with appropriate prerequisites. The two online theory courses are taught via computer on the World Wide Web technology through MSU's Virtual University. This system, which is compatible with both Windows and Mac platforms, is also supported by a 24-hour 7 days a week help desk. The laboratory component is typically taught over one week on MSU's East Lansing campus.

B) **Association for Molecular Pathology**

C) **Education from the Association for Molecular Pathology**

D) **American Society for Investigative Pathology JMD CME Program**

E) **American College of Medical Genetics Online Learning Center and CME Activities**

There are several conferences which focus on aspects of molecular testing. Further information will be available in forthcoming versions of this *Resource Guide*.

Section 10 Issues for the Practicing Pathologist

10.1 Billing and Reimbursement

A) Molecular Coding and Reimbursement
http://www.cap.org/apps/docs/practice_management/molecular_pathology_hcpcs_g_code.pdf

B) CAP Mobilizes For Accurate Molecular Pathology Payments
http://www.cap.org/apps/cap.portal?_nfpb=true&cntvwrPtlt_actionOverride=%2Fportlets%2FcontentViewer%2Fshow&_windowLabel=cntvwrPtlt&cntvwrPtlt%7BactionForm.contentReference%7D=statline%2Fstat031413.html&_state=maximized&_pageLabel=cntvwr#Story3

As disparate local Medicare pricing and coverage policies for the new molecular pathology molecular pathology CPT codes continue surfacing, CAP recently issued an appeal to pathology representatives from each state's Contractor Advisory Committee (CAC) to educate their Medicare contractors on national resource-based values and other supporting rationale developed when the new Molecular pathology codes were first proposed.

The CAP is encouraging all providers to give timely feedback to their Medicare contractor to assist them with establishing gap fill pricing for molecular pathology services. In addition, CAP is reminding pathologists of new rules for professional molecular pathology interpretation services provided to Medicare beneficiaries. The Medicare contractors have until April 1, 2013 to set their initial pricing for 2013 under Medicare's gap fill process.

Carrier Pricing Disparities
On November 1, 2012, the Centers for Medicare and Medicaid Services (CMS) announced that the molecular pathology codes developed through the AMA Current Procedural Terminology (CPT)

process would replace the existing molecular diagnostic “stacked” codes, and would be placed on the 2013 clinical laboratory fee schedule (CLFS) using the gap-fill pricing method.

With gap-fill or carrier pricing, local Medicare contractors are setting fees for 2013 based on local pricing patterns. In practice, reimbursement can vary significantly among carriers, and there can be inconsistency on which services are covered. After a year of each Medicare contractor setting its own pricing, CMS will set pricing for 2014 by calculating a national reimbursement rate for each code based on the combined local gap-fill amounts.

Gap-filled pricing is meant to take into account charges for the tests; discounts on the charges; required resources; payment amounts determined by other payers; and charges, resources, and payment amounts for tests that may be comparable. However, the CAP is concerned with the establishment of local payment rates by the individual Medicare contractors because it is unlikely that the individual contractors can duplicate the extensive, detailed and highly accurate process that the AMA Resource-Based Relative Value Scale Update Committee (RUC) used to recommend resource-based values for each molecular pathology code. These detailed direct cost analyses for each service, developed by CAP with AMP and other professional societies through the AMA RUC process, supersedes any other data source available for pricing these services. CAP believes that this data should be used to develop prices for the molecular Pathology codes.

Also, local coverage determinations may overlook the defined criteria used by the CPT Editorial Panel in establishing Category I CPT codes for each of these services, which require that:

- the service is consistent with current medical practice
- the clinical efficacy of the service is documented in literature that meets the requirements set forth in the CPT code change application
- the service is performed with frequency consistent with the intended clinical use

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For more information on the resources available for Molecular pathology coverage discussions, contact Nonda Wilson.

New G-code for Medicare Molecular Pathology Physician Interpretation

Read more about the MoPath G-code on the new CAP members-only online community—CAPconnect. Register now and join the conversation.

In a separate but related matter, CAP reaffirmed that Medicare physicians who interpret molecular tests and prepare written reports above and beyond laboratory results should already be billing for that service using the physician fee schedule (PFS) HCPCS G-code G0452 (molecular pathology procedure; physician interpretation and report).

CMS announced the creation of the G-code in the 2013 PFS, to be used when physician interpretation of a molecular pathology test is medically necessary to provide a clinically meaningful, beneficiary-specific result. However, there have been reports that some institutions still have not added the G-code G0452 to their billing system.

CMS' announcement in the 2013 final rule also included specific instruction and criteria associated with the new physician interpretation code:

> *This professional component-only HCPCS G-code will be considered a "clinical laboratory interpretation service" which is one of the current categories of PFS pathology services under the definition of physician pathology services at §415.130(b)(4).*
>
> *Section §415.130(b)(4) of the regulations and section 60 of the Claims Processing Manual (IOM 100-04, Ch. 12, section 60.E.) specify certain requirements for billing the professional component of certain clinical laboratory services including that the interpretation (1) must be requested by the patient's attending physician, (2) must result in a written narrative report included in*

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the patient's medical record, and (3) requires the exercise of medical judgment by the consultant physician.

We note that a hospital's standing order policy can be used as a substitute for the individual request by a patient's attending physician. The current CPT code for interpretation and report, 83912-26, is included on the current list of clinical laboratory interpretation services but will be deleted at the end of CY 2012.

For those providing molecular pathology physician interpretation services for Medicare beneficiaries who meet the above CMS criteria, G0452 should be reported.

"We encourage anyone performing these services to consult with your billing personnel to ensure the G-code is being used where appropriate," said Economic Affairs Committee Chair Jonathan Myles, MD, FCAP. "CMS has indicated that it would monitor utilization of the code and reassess the need for the code based on utilization. It's important to capture this information as accurately as possible."

Free text available from CAP's Statline Archives

C) Ins & Outs of Coding with the New Molecular Pathology CPT Procedure Codes AMP

Bossler AD, Nowak JA, C-coAEA Committee. Ins & Outs of Coding with the New Molecular Pathology CPT Procedure Codes AMP 2013 2/26/13

Summary: Ins & Outs of Coding with the New Molecular Pathology CPT Procedure Codes AMP Webinar Presented by the Co-Chairs of the AMP Economic Affairs Committee: Aaron D Bossler, MD, PhD and Jan A Nowak, MD, PhD on 2/26/13

Free text available from Association for Molecular Pathology

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10.2 Gene Patents

A) CAP Gene Patent/Supreme Court Decision Resource Center
http://www.cap.org/apps/cap.portal?_nfpb=true&cntvwrPtlt_actionOverride=%2Fportlets%2FcontentViewer%2Fshow&_windowLabel=cntvwrPtlt&cntvwrPtlt%7BactionForm.contentReference%7D=advocacy%2Fgene_patent_lawsuit_info_center.html&_state=maximized&_pageLabel=cntvwr

B) Patients Win in Supreme Court Gene Patent Decision
http://www.cap.org/apps/cap.portal?_nfpb=true&cntvwrPtlt_actionOverride=%2Fportlets%2FcontentViewer%2Fshow&_windowLabel=cntvwrPtlt&cntvwrPtlt%7BactionForm.contentReference%7D=media_resources%2Fnewsrel_SCOTUS_2013.html&_state=maximized&_pageLabel=cntvwr

The College of American Pathologists applauds today's unanimous Supreme Court decision invalidating the patents held by Myriad Genetics on the BRCA1 and BRCA2 genes. It is a victory for patients and for science.

CAP is a co-plaintiff in the case *Association of Molecular Pathology et al, vs Myriad Genetics, Inc.*, which the court decided today.

“This is a landmark decision,“ said CAP President, Stanley Robboy MD, FCAP. “Genomic medicine has the potential to be a cornerstone of medical testing, treatment, and clinical integration, but the question of “who owns your genes“ needed a definitive answer. Now we have it.“

The BRCA1 and BRCA2 genes are indicators for a hereditary predisposition to breast and ovarian cancer. It is thought that only 5% of women carry mutations in these genes that increase their cancer risk. Until the Court's decision today, a woman could only find out if they carry the mutated gene from a test provided by Myriad at a price of more than $3,000.

By invalidating Myriad's claims to human genes as well as all naturally occurring mutations of the genes, the Court opened the door for other

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companies and researchers who can now create their own tests and conduct their own research on the heretofore patented genes. Patient groups and medical groups have voiced concerns for several years that the patents stifled innovation, while the high cost of the tests made it difficult for many women to benefit from the tests that already exist.

“The Supreme Court decision invalidating Myriad Genetics' patents on BRCA1 and BRCA2, is a huge victory for patients," said Debra Leonard, MD, PhD, FCAP, Chair of CAP's Personalized Health Care Committee. "It will allow women to receive life saving, state-of-the-art genetic tests without being forced to trust one provider or one laboratory performing a single test to secure a diagnosis or inform treatment."

For more information on CAP's position on genomic medicine and on the case against Myriad Genetics, visit www.cap.org/advocacy.

Access CAP's Press Release

C) **Gene and Method Patents: Review of Court Cases and the Implications for Personalized Medicine**
Personalized Medicine Coalition Public Policy Committee Meeting, Gene and Method Patents: Review of Court Cases and the Implications for Personalized Medicine, December 6, 2011.

Access Personalized Medicine Coalitions Slides here

10.3 Ethical Considerations

A) **Privacy and Progress in Whole Genome Sequencing**
Presidential Commission for the Study of Bioethical Issues. Privacy and Progress in Whole Genome Sequencing. October, 2012. http://bioethics.gov/sites/default/files/PrivacyProgress508_1.pdf. Accessed September 26, 2013.

Free full text available

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B) Genomics, Health Care, and Society

Hudson KL. Genomics, health care, and society. *N Engl J Med.* 2011 Sep 15; 365(11):1033–1041.

Summary: A new generation of genomic technologies permits the increased collection of data on large study populations. New methods in informatics facilitate the integration of diverse types of information with genomic data in disease research. As a result, researchers are learning more about the genetic bases of disease and response to drugs. Genetic tests, including many that are offered directly to the consumer, are growing in number and clinical relevance. Genomic knowledge and technologies are also being adopted in areas distant from human health. Here, I describe evolving policies pertinent to genetic and genomic research, the integration of genetics into clinical care, and the broader issues raised by genetic technologies and information.

Free full text available from *New England Journal of Medicine*
PMID: 21916641

C) Preparing for a Consumer-Driven Genomic Age

Evans JP, Dale DC, Fomous C. Preparing for a consumer-driven genomic age. *N Engl J Med*. 2010 Sep 16; 363(12):1099–1103.

Summary: Advances in genomic technologies permit the simultaneous analysis of millions of variants across the genome and may soon allow for meaningful estimation of one's risks of developing cancer, diabetes, and other common diseases. These advances are converging with the movement toward consumer-driven health care and patient empowerment. Whereas in the past, medical testing was firmly under the control of medical practitioners, genomic information is now increasingly available outside traditional medical settings. Patients are no longer subordinate, passive recipients of physician-initiated genetic testing; rather, patients can instigate their own testing and often know more than their clinicians about particular genetic topics. Indeed, health care providers are increasingly bypassed altogether, as patients embrace direct-to-consumer (DTC) genetic tests and turn to social networks for help in interpreting their results. In the future, a primary role of health care professionals may be to

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interpret patients' DTC genetic test results and advise them about appropriate follow-up. How can we maximize the benefits of these new developments and minimize the harms? How can we encourage patients' involvement and autonomy yet establish appropriate safeguards while avoiding inappropriate paternalism? How do we promote the understanding that interpretations of genomic information may evolve as research unravels the meaning of gene–gene and gene–environment interactions and the roles of noncoding DNA sequences, copy-number variants, epigenetic mechanisms, and behavioral factors in health and disease?

Free full text available from *New England Journal of Medicine*
PMID: 20843241

D) Beyond the HIPAA Privacy Rule: Enhancing Privacy, Improving Health Through Research. 2009

Institute of Medicine (US) Committee on Health Research and the Privacy of Health Information: The HIPAA Privacy Rule; Nass SJ, Levit LA, Gostin LO, editors. Beyond the HIPAA Privacy Rule: Enhancing Privacy, Improving Health Through Research. 2009. Washington (DC): National Academies Press (US); 2009.

Summary: The U.S. Department of Health and Human Services (HHS) developed a set of federal standards for protecting the privacy of personal health information under the Health Insurance Portability and Accountability Act of 1996 (HIPAA). The HIPAA Privacy Rule set forth detailed regulations regarding the types of uses and disclosures of individuals' personally identifiable health information-called "protected health information"-permitted by "covered entities" (health plans, health care clearinghouses, and health care providers who transmit information in electronic form in connection with transactions for which HHS has adopted standards under HIPAA). A major goal of the HIPAA Privacy Rule is to ensure that individuals' health information is properly protected while allowing the flow of information needed to promote high-quality health care. The HIPAA Privacy Rule also set out requirements for the conduct of health research. The Institute of Medicine Committee on Health Research and the Privacy of Health Information (the committee) was charged with two principal tasks : (1) to assess whether the HIPAA Privacy Rule is having an

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impact on the conduct of health research, defined broadly as "a systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge" ; and (2) to propose recommendations to facilitate the efficient and effective conduct of important health research while maintaining or strengthening the privacy protections of personally identifiable health information.

Free full text available from *National Academies Press*
PMID: 20662116

E) AMA Summary of the Health Insurance Portability and Accountability Act (HIPAA) Omnibus Final Rule Summary
American Medical Association. AMA summary of the Health Insurance Portability and Accountability Act (HIPAA) Omnibus Final Rule Summary. http://www.ama-assn.org/resources/doc/washington/hipaa-omnibus-final-rule-summary.pdf. Accessed September 26, 2013.

Summary: The federal government has published its long awaited final regulations implementing the “Health Information Technology for Economic and Clinical Health (HITECH) Act,” enacted as part of the “American Recovery and Reinvestment Act of 2009” (ARRA), described by the head of the Office for Civil Rights (OCR) in the Department of Health and Human Services (HHS) as “the most sweeping changes to the HIPAA Privacy and Security Rules since they were first implemented.” In general, the new rules expand the obligations of physicians and other health care providers to protect patients’ protected health information (PHI), extend these obligations to a host of other individuals and companies who, as “business associates,” have access to PHI, and increase the penalties for violations of any of these obligations. The American Medical Association (AMA) will be publishing more detailed guidance concerning the impact of these rules on physicians. The following outlines the changes physicians will need to consider as they implement the new HIPAA requirements necessary by the September 23, 2013, compliance date.

Free full text available from American Medical Association

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10.4 Validation

A) Recommended Principles and Practices for Validating Clinical Molecular Pathology Tests

Jennings, L, Van Deerlin VM, Gulley ML, College of American Pathologists Molecular Pathology Resource Committee. Recommended principles and practices for validating clinical molecular pathology tests. *Arch Pathol Lab Med.* 2009 May; 133(5): 743-755.

Summary: The use of DNA- and RNA-based tests continues to grow for applications as varied as inherited disease, infectious disease, cancer, identity testing, human leukocyte antigen typing, and pharmacogenetics. Progress is driven in part by the huge growth in knowledge about the molecular basis of disease coupled with advancements in technologic capabilities. In addition to requirements for clinical utility, every molecular test also may have limitations that must be carefully considered before clinical implementation. Analytic and clinical performance characteristics as well as test limitations are established and documented through the process of test validation. OBJECTIVE: To describe the established principles of test validation, along with relevant regulations in the United States, in order to provide a rational approach to introducing molecular tests into the clinical laboratory. DATA SOURCES: PubMed review of published literature, published guidelines, and online information from national and international professional organizations. CONCLUSIONS: These resources and recommendations provide a framework for validating clinical tests.

Free full text available from the CAP's *Archives*
PMID: 19415949

B) A Standardized Framework for the Validation and Verification of Clinical Molecular Genetic Tests

Mattocks CJ, Morris MA, Matthijs G, et al. A standardized framework for the validation and verification of clinical molecular genetic tests. *Eur J Hum Genet.* 2010 Dec; 18(12): 1276-1288.

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Summary: The validation and verification of laboratory methods and procedures before their use in clinical testing is essential for providing a safe and useful service to clinicians and patients. This paper outlines the principles of validation and verification in the context of clinical human molecular genetic testing. We describe implementation processes, types of tests and their key validation components, and suggest some relevant statistical approaches that can be used by individual laboratories to ensure that tests are conducted to defined standards.

Free full text available from PubMed
PMID: 20664632

C) Validation of Fluorescence In Situ Hybridization Using an Analyte-Specific Reagent for Detection of Abnormalities Involving the Mixed Lineage Leukemia Gene

Saxe DF, Persons DL, Wolff DJ, Theil KS; Cytogenetics Resource Committee of the College of American Pathologists. Validation of fluorescence in situ hybridization using an analyte-specific reagent for detection of abnormalities involving the mixed lineage leukemia gene. *Arch Pathol Lab Med.* 2012 Jan; 136(1): 47-52.

Summary: Fluorescence in situ hybridization (FISH) is a molecular cytogenetic assay that is commonly used in laboratory medicine. Most FISH assays are not approved by the US Food and Drug Administration but instead are laboratory-developed tests that use analyte-specific reagents. Although several guidelines exist for validation of FISH assays, few specific examples of FISH test validations are available in the literature. OBJECTIVE: To provide an example of how a FISH assay, using an analyte-specific reagent probe, may be validated in a clinical laboratory. DESIGN: We describe the approach used by an individual laboratory for validation of a FISH assay for mixed lineage leukemia (MLL) gene. RESULTS: Specific validation data are provided illustrating how initial assay performance characteristics in a FISH assay for MLL may be established. CONCLUSIONS: Protocols for initial validation of FISH assays may vary between laboratories. However, all laboratories must establish several defined performance specifications prior to implementation of FISH assays for clinical use. We describe an approach used for

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assessing performance specifications and validation of an analyte-specific reagent FISH assay using probes for MLL rearrangement in interphase nuclei.

Free full text available from the CAP's *Archives*
PMID: 22208487

D) College of American Pathologists Proposal for the Oversight of Laboratory-Developed Tests

Vance G. College of American Pathologists Proposal for the Oversight of Laboratory-Developed Tests. *Arch Pathol Lab Med*. 2011;135:1432–1435.

Summary: Context.—Fluorescence in situ hybridization (FISH) is a molecular cytogenetic assay that is commonly used in laboratory medicine. Most FISH assays are not approved by the US Food and Drug Administration but instead are laboratory-developed tests that use analyte-specific reagents. Although several guidelines exist for validation of FISH assays, few specific examples of FISH test validations are available in the literature. Objective.—To provide an example of how a FISH assay, using an analyte-specific reagent probe, may be validated in a clinical laboratory. Design.—We describe the approach used by an individual laboratory for validation of a FISH assay for mixed lineage leukemia (MLL) gene. Results.—Specific validation data are provided illustrating how initial assay performance characteristics in a FISH assay for MLL may be established. Conclusions.—Protocols for initial validation of FISH assays may vary between laboratories. However, all laboratories must establish several defined performance specifications prior to implementation of FISH assays for clinical use. We describe an approach used for assessing performance specifications and validation of an analyte-specific reagent FISH assay using probes for MLL rearrangement in interphase nuclei.

Free full text available from the CAP's *Archives*

E) Modernizing US Regulatory and Reimbursement Policy to Support Continued Innovation in Genomic Pathology

Walcoff SD, Pfeifer JD. Modernizing US regulatory and

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reimbursement policy to support continued innovation in genomic pathology. Personalized Medicine 2012; 9:295-308.

Summary: The pace of technical and scientific advancement for genomics-based technologies has outstripped the ability of the US regulatory bodies to keep abreast of the emerging paradigms, resulting in regulatory pronouncements that often appear dated and payment systems that are misaligned. Over burdensome evidentiary requirements, encroachment of federal regulators into the practice of laboratory medicine, and failure to align public health objectives with pay or valuation sufficient to support professional pathology services and necessary capital investment threaten to stifle continued innovation in genomic-based diagnostic tools. Nevertheless, the US FDA is committed to improving regulatory science and to increased stakeholder participation in policy-making, and serious efforts to address coding and test value are underway. Moreover, federal legislation will be debated in the coming months that, if enacted, could clarify authorities and institute meaningful regulatory and reimbursement paradigms better suited to molecular testing. This review explores these dynamic policy issues and their implications for genomic pathology as the foundation of personalized medicine.

Full text available from *Personalized Medicine* (USD 60.00 for 24 hour access)

F) Design and Analytical Validation of Clinical DNA Sequencing Assays

Pont-Kingdon G, Gedge F, Wooderchak-Donahue W, et al. Design and analytical validation of clinical DNA sequencing assays. *Arch Pathol Lab Med.* 2012 Jan; 136(1): 41-46.

Summary: CONTEXT: DNA sequencing is the method of choice for mutation detection in many genes. OBJECTIVES: To demonstrate the analytical accuracy and reliability of DNA sequencing assays developed in clinical laboratories. Only general guidelines exist for the validation of these tests. We provide examples of assay validation strategies for DNA sequencing tests. DESIGN: We discuss important design and validation considerations. RESULTS: The validation examples include an accuracy study to evaluate concordance

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between results obtained by the newly designed assay and analyzed by another method or laboratory. Precision (reproducibility) studies are performed to determine the robustness of the assay. To assess the quality of sequencing assays, several sequence quality measures are available. In addition, assessing the ability of primers to specifically and robustly amplify target regions before sequencing is important. CONCLUSION: Protocols for validation of laboratory-developed sequencing assays may vary between laboratories. An example summary of a validation is provided.

Free full text available from the CAP's *Archives*
PMID: 22208486

G) Design and Analytic Validation of BCR-ABL1 Quantitative Reverse Transcription Polymerase Chain Reaction Assay for Monitoring Minimal Residual Disease

Jennings LJ, Smith FA, Halling KC, Persons DL, Kamel-Reid S,Molecular Oncology Resource Committee of the College of American P. Design and analytic validation of BCR-ABL1 quantitative reverse transcription polymerase chain reaction assay for monitoring minimal residual disease. *Arch Pathol Lab Med.* 2012 Jan; 136(1): 33-40.

Summary: CONTEXT: Monitoring minimal residual disease by quantitative reverse transcription polymerase chain reaction has proven clinically useful, but as yet there are no Food and Drug Administration-approved tests. Guidelines have been published that provide important information on validation of such tests; however, no practical examples have previously been published. OBJECTIVE: To provide an example of the design and validation of a quantitative reverse transcription polymerase chain reaction test. DESIGN: To describe the approach used by an individual laboratory for development and validation of a laboratory-developed quantitative reverse transcription polymerase chain reaction test for BCR-ABL1 fusion transcripts. RESULTS: Elements of design and analytic validation of a laboratory-developed quantitative molecular test are discussed using quantitative detection of BCR-ABL1 fusion transcripts as an example. CONCLUSIONS: Validation of laboratory-developed quantitative molecular tests requires careful planning and execution to

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adequately address all required analytic performance parameters. How these are addressed depends on the potential for technical errors and confidence required for a given test result. We demonstrate how one laboratory validated and clinically implemented a quantitative BCR-ABL1 assay that can be used for the management of patients with chronic myelogenous leukemia.

Free full text available from the CAP's *Archives*
PMID: 22208485

H) Validation of KRAS Testing for Anti-EGFR Therapeutic Decisions for Patients with Metastatic Colorectal Carcinoma

Kamel-Reid S, Zhang T, Persons DL, Nikiforova MN, Halling KC,Molecular Oncology Resource Committee of the College of American P. Validation of KRAS testing for anti-EGFR therapeutic decisions for patients with metastatic colorectal carcinoma. *Arch Pathol Lab Med.* 2012 Jan; 136(1): 26-32.

Summary: CONTEXT: KRAS mutation status is a molecular marker for predicting patient response to treatment with anti-EGFR antibodies (cetuximab and panitumumab) in metastatic colorectal carcinoma. Different approaches may be taken to detect KRAS mutations. There currently are no US Food and Drug Administration-approved assays for the detection of KRAS mutations. For assays that are not approved by the US Food and Drug Administration, the performance characteristics of the assay must be determined and validated by the clinical laboratory before implementation. OBJECTIVE: To provide an example of how a KRAS mutation-analysis assay may be validated in a clinical laboratory. DESIGN: Describing the approach used by an individual laboratory to compare different assays for validation of KRAS mutation analysis in metastatic colon carcinoma. RESULTS: Specific validation data are provided, illustrating how a laboratory established assay performance characteristics for KRAS mutation analysis. CONCLUSIONS: All clinical laboratories must establish several performance specifications mandated by the Clinical Laboratory Improvement Amendments of 1988 before implementation of any laboratory-developed test. Approaches to the validation of such assays may vary among laboratories. We describe an approach used for validation of a KRAS mutation-analysis assay by one laboratory.

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PMID: 22208484

I) Verification of Performance Specifications of a Molecular Test: Cystic Fibrosis Carrier Testing Using the Luminex Liquid Bead Array

Lacbawan FL, Weck KE, Kant JA, et al. Verification of performance specifications of a molecular test: cystic fibrosis carrier testing using the Luminex liquid bead array. *Arch Pathol Lab Med.* 2012 Jan; 136(1): 14-19.

Summary: CONTEXT: The number of clinical laboratories introducing various molecular tests to their existing test menu is continuously increasing. Prior to offering a US Food and Drug Administration-approved test, it is necessary that performance characteristics of the test, as claimed by the company, are verified before the assay is implemented in a clinical laboratory. OBJECTIVE: To provide an example of the verification of a specific qualitative in vitro diagnostic test: cystic fibrosis carrier testing using the Luminex liquid bead array (Luminex Molecular Diagnostics, Inc, Toronto, Ontario). DESIGN: The approach used by an individual laboratory for verification of a US Food and Drug Administration-approved assay is described. RESULTS: Specific verification data are provided to highlight the stepwise verification approach undertaken by a clinical diagnostic laboratory. CONCLUSIONS: Protocols for verification of in vitro diagnostic assays may vary between laboratories. However, all laboratories must verify several specific performance specifications prior to implementation of such assays for clinical use. We provide an example of an approach used for verifying performance of an assay for cystic fibrosis carrier screening.

Free full text available from the CAP's *Archives*
PMID: 22208482

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Section 11 Feedback

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How to Cite This Document

Santoro S, Arnaout R, Burton MP, Coleman JF, Cushman-Vokoun A, Grossmann A, Kaufman JH, Kiechle FL, Laser JS, Mullins FM. CAP Pathology Resource Guide: Molecular Pathology. Version 5.0(1). Northfield, IL: College of American Pathologists; 2014.

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The CAP Pathology Resource Guide: Molecular Pathology was updated by CAP's Personalized Health Care Committee.

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