Technology Assessment

Technology Assessment Program

Quality, Regulation and Clinical Utility of Laboratory-developed Molecular Tests

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This report is based on research conducted by the ECRI Institute Evidence-based Practice Center under contract to the Agency for Healthcare Research and Quality (AHRQ), Rockville, MD (Contract Number 290 2007 10063 I).The findings and conclusions in this document are those of the author(s) who are responsible for its contents; the findings and conclusions do not necessarily represent the views of AHRQ. No statement in this article should be construed as an official position of the Agency for Healthcare Research and Quality or of the U.S. Department of Health and Human Services.

The information in this report is intended to help health care decision-makers; patients and clinicians, health system leaders, and policymakers, make well-informed decisions and thereby improve the quality of health care services. This report is not intended to be a substitute for the application of clinical judgment. Decisions concerning the provision of clinical care should consider this report in the same way as any medical reference and in conjunction with all other pertinent

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One of the investigators, Raylene Ballard, serves as an Interdisciplinary Liaison for the American Association for Clinical Chemistry. Dr. Wendy Bruening worked from 1990 – 1992 as a member of the team that discovered the Wilms' tumor suppressor gene (WT1) while she was an undergraduate student at Massachusetts Institute of Technology (MIT). She receives royalties from MIT related to the patent filed by the university for the gene sequence, but has no control over uses of the gene sequence, and no conflict with the material in this report. None of the other investigators has any affiliations or financial involvement related to the material presented in this report.

Peer Reviewers

We wish to acknowledge individuals listed below for their review of this report. This report has been reviewed in draft form by individuals chosen for their expertise and diverse perspectives. The purpose of the review was to provide candid, objective, and critical comments for consideration by the EPC in preparation of the final report. Synthesis of the scientific literature presented here does not necessarily represent the views of individual reviewers.

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Table of Contents

Tables

Figure

Introduction

The Coverage and Analysis Group at the Centers for Medicare & Medicaid Services (CMS) requested from The Technology Assessment Program (TAP) at the Agency for Healthcare Research and Quality (AHRQ) a horizon scan to summarize the available scientific evidence on the quality of laboratory-developed ("home brew" or "in-house") molecular tests, which are currently not actively regulated by the U.S. Food and Drug Administration (FDA). CMS has concerns about the quality of laboratory-developed tests and the validation currently being performed on these tests. AHRQ assigned this report to the following Evidence-based Practice Center (EPC): ECRI EPC (Contract Number: 290 2007 10063 I). To help CMS to address its concerns, this horizon scan is intended to: 1) identify types of laboratory-developed molecular tests (LDMTs) currently available for conditions relevant to the Medicare over-65-year-old population, 2) identify the methodologies and the processes that have been developed for the assessment of analytical and clinical performance of molecular tests, 3) summarize the role of Federal agencies in regulating LDMTs, and 4) identify the quality standards that have been developed for molecular tests by regulatory bodies, the industry, and the medical community.

Scope of this Report

In this report, we use the term "molecular test" (MT) interchangeably with the term "molecular genetic test." We adopted the definition of molecular genetic test recommended by the Genetic Work Group of the Clinical Laboratory Improvement Advisory Committee. The Work Group defined a genetic molecular test as "an analysis performed on human DNA or RNA to detect heritable or acquired disease-related genotypes, mutations, or phenotypes for clinical purposes."[1] According to this definition, cytogenetic tests, which are performed on human chromosomes, and biochemical genetic tests, which analyze human proteins and certain metabolites, are beyond the scope of this report. However, molecular cytogenetic tests (e.g., the tests using the fluorescence in situ hybridization (FISH) technology) in which analyses cross both the chromosome and the DNA levels are included in this report. In this report, we also consider a test performed on pathogen (e.g., bacterial, viral or fungal) DNA or RNA as a molecular test if the purpose of the test is to diagnose an infectious disease caused by the pathogen in human.

In accordance with the objectives outlined in the scope of work, this horizon scan only includes molecular tests of potential clinical relevance to the Medicare over-65-year-old population as of October 31, 2008. Particularly, molecular tests for the following purposes are addressed:

- tests used for diagnostic purposes in symptomatic individuals,
- tests used as prognostic indicators,
- tests used to monitor response to therapy, and
- tests used to choose therapies for a known disease entity or used to adjust medication dosing.

Excluded from this report are molecular tests used primarily for blood supply screening, tissue typing, epidemiological surveillance, pure research, and forensic purposes. Tests used to screen for inherited diseases of metabolism or other conditions of greater relevance to the pediatric population (e.g., the diagnostic or screening tests for cystic fibrosis) are also beyond the scope of this report.

A molecular test can be performed using either a protocol developed within the laboratory or a test kit developed by a manufacturer for commercial distribution to multiple laboratories. Commercially distributed test kits include all reagents and instructions needed to complete the test procedure and interpret the results. These commercial kits are currently regulated by the FDA as in-vitro diagnostic devices. Laboratory-developed molecular tests (LDMTs), also known as homebrew or in-house molecular tests, are developed within laboratories using either FDA regulated or self-developed analyte specific reagents (ASRs) and intended for use solely in the test developer's laboratory. LDMTs are not actively regulated by the FDA, although the Agency claims its jurisdiction over such tests. This report focuses on LDMTs; however, in cases where we cannot tell whether a test offered by a laboratory uses a commercial kit or is one developed in house, we included the test and labeled it differently from those clearly identified as LDMTs.

Overview of Molecular Testing Technology

During the last few decades, advances in genetic science have greatly extended and deepened the understanding of the mechanisms of diseases at the molecular level. This understanding is now being translated into diagnostic, prognostic, and therapeutic tools, and molecular tests have now been developed for a wide variety of clinical conditions.

One of the most popular applications of molecular tests (as we catalog in Chapter 1 of this report) is to detect the presence of particular viruses, bacteria, or other types of pathogens in samples taken from patients. The molecular methods are often faster and more sensitive than traditional microbiological diagnostic techniques such as cultures, antigen detection, and microscopic visualization. Molecular testing is particularly valuable for detecting microorganisms that cannot be easily cultured, require long incubation, or are not easily recovered for technical reasons (specimen transport requirements, prolonged turnaround times, technically demanding procedures, etc.). Molecular tests can also be used to quantify the loads of detected microorganisms, which can be useful in assessing disease severity and monitoring treatment efficacy.

Molecular tests are also widely used in oncology and hematology. As Chapter 1 of this report shows, various malignancy-associated molecular tests have been developed to aid diagnosis, to evaluate prognosis, to detect residual or recurrent disease, and to aid in selecting treatment. For example, molecular tests to detect the *ERBB2* gene (also known as *HER2*) have gained acceptance as a potentially useful aid in customizing treatment strategies for breast cancer. Detection of particular fusion transcripts (a type of genetic marker) in the blood by polymerase chain reaction (PCR), a genetic technique discussed below, has been used in the diagnosis and management of hematological malignancies. FISH (another genetic technique that is discussed later) has also been used to study chromosomal abnormalities in both hematological malignancies and solid tumors.

Molecular techniques are also used outside of the infectious disease and malignancy areas. For example, research has shown that some of the unexpected response to a blood-thinning drug,

warfarin (Coumadin®), depends on variants of two genes: *CYP2C9* and *VKORC1*. The FDA has cleared several molecular test kits for detecting such genetic variants. These molecular tests may potentially aid physicians in choosing the right dosage of warfarin for patients. In addition, molecular tests are widely used in blood supply screening, tissue typing, epidemiological surveillance, forensic testing, and heritable disease screening, although these applications are beyond the scope of this report.

Major Molecular Techniques

Over the years, a number of techniques have been developed for molecular testing. To facilitate the discussions in the coming chapters, we provide a brief overview of two groups of molecular techniques most frequently used in the tests that we cataloged for this report.

Polymerase Chain Reaction (PCR)

PCR is a nucleic acid amplification technique widely used in molecular testing. During PCR, the sample containing the DNA pieces for testing (targets) is heated first so that the two strands of the DNA become separated (a process called denaturation). Then, primers—short nucleic acid strands that are complementary to the targets—are used to bind with the targets on each separated DNA strand (a process called annealing). Once primers are annealed to the targeted DNA fragments, a special enzyme called DNA polymerase will start to catalyze the DNA replication process. Each PCR cycle doubles the amount of the targeted DNA sequences. After multiple PCR cycles, a single (or a few fragments of a) DNA strand is exponentially amplified (replicated) into millions or more copies within a short span of time. The PCR technique allows selective isolation and amplification of specific DNA fragments thus permitting methods of analysis that require large amounts of a particular fragment of DNA (e.g., DNA sequencing, genetic fingerprinting, hybridization).

Over the years, the basic PCR technique has been modified to perform a wide array of genetic manipulations and diagnostic tests. As a result, various derivatives of the technique have emerged. For example, reverse transcription PCR (RT-PCR) was developed to detect and quantify messenger RNA (mRNA) sequences to study gene expression within a cell or tissue. During RT-PCR, the RNA strand is first transcribed reversely into its complementary DNA (cDNA), and then the PCR technique is used to amplify the resulting cDNA. Another popular PCR derivative, multiplex PCR, uses multiple, unique primer sets simultaneously to detect multiple targets, which greatly improves testing efficiency. Also a derivative of the basic PCR technique, real-time PCR allows quantitative estimation of the amount of the DNA sequence of interest present in a sample. Since real-time PCR can amplify and quantify targeted DNA or RNA sequences simultaneously throughout the testing process, it significantly reduces test turnaround time.

In addition to those mentioned above, other PCR-based test methods (e.g., allele-specific PCR, assembly PCR, asymmetric PCR, colony PCR, hot-start PCR, intersequence-specific PCR, Inverse PCR, Ligation-mediated PCR, Methylation-specific PCR, nested PCR, overlap-extension PCR, TAIL-PCR, and touchdown PCR), as well as PCR-like nucleic acid amplification techniques (e.g., ligase chain reaction, helicase-dependent amplification and multiplex ligation-dependent probe amplification), have also been developed and are used for clinical or research purposes. PCR (including PCR-based methods and PCR-like nucleic acid amplification techniques) are the most frequently used molecular methods in the tests that we cataloged for this report (see Chapter 1).

Hybridization

Hybridization is a molecular technique that involves detection of specific DNA or RNA sequences by hybridizing (i.e., binding or annealing) a labeled probe—a nucleic acid sequence complementary to the target of interest—to the DNA. Several in situ hybridization methods including fluorescence in situ hybridization (FISH), chromogenic in situ hybridization (CISH), and silver in situ hybridization (SISH) have been developed to detect and localize specific DNA sequences on chromosomes. During in situ hybridization, thin slices of a sample tissue are cut and attached to slides, or blood samples are smeared onto slides. The DNA in the cells is denatured by chemicals or heat. Then a piece of labeled DNA probe (in FISH, e.g., labeled fluorescently) is washed across the slide and hybridizes to the exposed gene on the denatured DNA strand by complementary base pairs. After the hybridization procedure, the slides are examined (in FISH, e.g., under a fluorescent microscope) for detection and localization of the gene of interest on the chromosomes. In situ hybridization techniques have been used in many malignancy-related molecular tests that we cataloged for this report. The technique can also be used in some infectious disease testing where the probe binds to the viral DNA/RNA or bacterial material.

Microarray (sometimes referred to as DNA chip) is another hybridization technique that has been used in a small number of tests that we cataloged. This technique allows simultaneous detection of multiple targets (mRNA or DNA sequences) from the same sample within a short span of time. Microarrays are produced by spotting probes (small fragments of DNA complementary to targets) onto the support medium (e.g., glass microscope slides, silicon chips, or nylon membranes) according to a predetermined pattern. During a test using microarrays, targets in the sample hybridize to the probes and the pattern of hybridization is then evaluated. One of the major applications of the microarray technology is to assay gene expression. Using microarrays, scientists can determine the expression levels of genes within a cell by measuring the amount of mRNA bound to each site on the array. Another popular application of microarrays is analysis of single nucleotide polymorphisms (SNP) that are DNA sequence variations that may be associated with susceptibility to disease or related to drug responsiveness.

The hybridization technique is also used as an element in other molecular methods such as Southern blot, a technique to check for the presence of a DNA sequence in a DNA sample, and Northern blot, a technique for gene expression study. Those techniques use a labeled probe to hybridize to the targeted DNA or RNA sequence in the late stage of the test so that the presence or absence of the targeted sequence can be detected.

Challenges in Molecular Test Assessment and Oversight

With the continuous advances in genetic research and molecular technologies, especially with the completion of the Human Genome Project, molecular diagnostic testing has become a fast-growing service area. Many clinical laboratories or diagnostic test kit manufacturers have entered, or are entering, this potentially lucrative market. For this report alone, we cataloged over 1,400 molecular tests relevant to the Medicare over-65-year-old population (as of October 31, 2008) offered by 95 different laboratories. Chapter 2 provides a detailed listing and discussion of these tests. Note that our catalogue is not all-inclusive, e.g., tests primarily used in the pediatric population or for heritable conditions are not included.

As with any other diagnostic test, molecular tests, whether they are laboratory-developed or commercial kits, can be evaluated at multiple levels. While no consensus has been reached on

any of the currently proposed frameworks for the evaluation of molecular tests, many experts in the field argued that such evaluation should cover several key components, including the tests' analytic validity, clinical validity, and clinical utility.[2,3] Analytic validity simply refers to how well a test performs in the laboratory—how well does the test measure the properties or characteristic it is intended to measure (e.g., a gene mutation)? Clinical validity (also known as diagnostic accuracy) refers to the accuracy with which a test predicts the presence or absence of a clinical condition or predisposition. Clinical utility refers to the usefulness of the test and the value of information to medical practice. If a test has utility, it means that the results of the test can be used to pursue effective treatment or provide other concrete benefit. Chapters 2, 3 and 7 of this report describe how the analytic validity, clinical validity, and clinical utility of molecular tests are assessed.

The oversight of laboratory tests in the U.S. is provided by a still-evolving system that currently includes Government agencies, health care payers, professional associations, and other stakeholders. (A recent report published by the Secretary's Advisory Committee on Genetics, Health, and Society [SACGHS] provided an overview of the system).[3] At the Federal level, the FDA regulates commercially distributed test kits as in-vitro diagnostic devices. However, the majority of molecular tests are laboratory-developed tests (LDTs). LDTs are not actively regulated by the FDA, although the Agency claims its jurisdiction over such tests and currently regulates the commercially distributed ASRs used in LDMTs.

A laboratory that performs tests of moderate or high technical complexity (including most, if not all, molecular tests) is subject to the regulations of the Clinical Laboratory Improvement Amendments (CLIA). Under the current CLIA regulations, the analytic validity of the tests is the primary focus of the assessment. CLIA leave the assessment responsibility for clinical relevance of LDMTs mostly to the directors or clinical consultants of the laboratories that provide the testing service. Although the assessment of clinical relevance of the tests is required to be documented and reviewed in the CLIA certification process, the result of the assessment is rarely available to the public or decision makers for review.

Meanwhile, unlike most of the other tests of moderate or high complexity, molecular tests do not have a CLIA-designated specialty or sub-specialty of their own. No formal CLIA-approved proficiency testing (PT) programs (i.e., external test quality control programs) have been established for molecular tests. Laboratories are currently required to use alternative methods to validate the analytical performance of molecular tests prior to offering them to patients (e.g., through a sample split program or an unofficial PT program). It is still unclear whether the alternative validation methods are as effective as a formal proficiency testing program in detecting potential quality problems.

The current oversight status (the details of which are described in chapters 4, 5 and 6 of this report) has generated concern among the public and medical community alike about the quality of LDMTs. The complex nature of molecular tests further intensifies such concerns. As we discuss in Chapters 2, 3, and 7, many technical problems may occur in the complex molecular testing processes, such as flawed probe, primer, or array design. Recently, the Clinical Laboratory Improvement Advisory Committee (CLIAC) Genetic Testing Good Laboratory Practices Workgroup published a report that provided a series of recommendations for ensuring the quality of molecular genetic testing.[4] These recommendations were made to the CLIAC, an advisory entity that has been providing recommendations to the Department of Health and Human Services on approaches needed to ensure the quality of genetic testing since 1997.

Key Questions and Organization of this Report

This Horizon Scan addresses the following eight Key Questions:

1. What types of laboratory-developed molecular tests are currently available for conditions relevant to the Medicare over-65-year-old population?
2. How is analytic validity established for laboratory-developed molecular tests?
3. What processes have been developed for examining clinical validity and clinical utility of molecular tests?
4. How are molecular tests regulated by the Clinical Laboratory Improvement Amendments (CLIA)?
5. What Food and Drug Administration (FDA) guidance has been issued pertaining to oversight of laboratory-developed molecular testing?
6. What is the role of other Federal agencies (e.g., Federal Trade Commission) in regulating marketing claims regarding the clinical validity and utility of laboratory-developed tests not currently being actively regulated by the FDA?
7. How is proficiency testing accomplished for molecular tests, whether laboratory-developed or commercial?
8. What guidelines and standards exist for laboratories conducting molecular testing?

Based on the Key Questions, this report consists of the *Introduction*, 8 chapters (each addressing one question) and an *Epilogue*. The methods and results for each Key Question are described in each chapter. Unless specified otherwise, the literature search strategy for each Key Question is provided in Appendix A of this report.

Chapter 1. What Types of Laboratory-Developed Molecular Tests Are Currently Available for Conditions Relevant to the Medicare Over-65-Year-Old Population?

For Key Question 1, we were asked to identify and catalogue laboratory-developed molecular tests (LDMTs) currently being performed that may be relevant to the Medicare over-65-year-old population. Particularly, we were asked to include tests used for the following purposes:

- to confirm diagnosis in symptomatic individuals;
- to choose therapies for a known disease entity or to adjust medication dosing;
- as prognostic indicators; or
- to make treatment decisions and monitor therapy.

As we described in the previous chapter, we excluded tests used primarily for forensic purposes, to screen donated blood for infectious diseases, to screen for inherited diseases of metabolism or other conditions of greater relevance to the pediatric population, and tests for which there are currently no clinical (diagnostic, prognostic or therapeutic) applications (i.e., tests used primarily for research purposes).

Methods

To identify LDMTs, we considered scientific literature published in peer-reviewed journals and other publications, giving highest priority to systematic reviews, followed by other publication types, including evaluation studies. We also consulted a variety of gray literature sources, including laboratory Web sites and accrediting organization Web sites, such as the College of American Pathologists (CAP) and the Association for Molecular Pathology (AMP). For this question, we found that the most useful and efficient method of identifying LDMTs was through the AMP Web site (www.amptestdirectory.org).

The AMP is a not-for-profit scientific society founded in 1995[5], that is dedicated to the advancement, practice, and science of clinical molecular laboratory medicine and translational research.[6] AMP membership includes laboratories from academia, government, and industry.[5] The AMP members voluntarily list their laboratories and research or clinical molecular tests for inclusion in the AMP test directory. Because membership and listing is voluntary, the AMP test directory is not inclusive of all laboratories or available tests.

Within the AMP Web site, molecular tests are categorized according to the following three conditions: infectious diseases, solid tumors, and hematopathology. The information available from the AMP online test directory is presented in Table 11 in Appendix B. We used the AMP Web site as a starting point for identifying and cataloging molecular tests. This Web site contains information about molecular tests available from most of the laboratories identified in the Statement of Work (SOW) as relevant sources for this report, which include the following:

- Armed Forces Institute of Pathology
- ARUP Laboratories
- Barnes and Jewish Hospital Molecular Diagnostics Laboratory

- Duke University Medical Center
- Mayo Medical Laboratories
- MD Anderson Molecular Diagnostic Laboratory
- Oregon Health and Sciences University
- Targeted Diagnostics and Therapeutics Inc.
- University of Nebraska
- University of Pennsylvania
- University of Utah
- Upstate Medical University
- Vanderbilt University Medical Center
- Yale University

The AMP directory also contains information about available molecular tests from 63 additional laboratories. The AMP test directory did not, however, include test information from the following three commercial diagnostic laboratories identified in the SOW: Quest Diagnostics, LabCorp, and Specialty Labs. To obtain information about tests available through these laboratories, we consulted the individual laboratory Web sites. We assumed, unless otherwise specified, that the tests listed in their comprehensive test catalogues were developed by the individual laboratory.

We also searched the Web sites of two other organizations that collect information about available molecular tests—GENDIA and Genetest. GENDIA (for GENetic DIAgnostics) consists of a network of international laboratories performing molecular genetic testing in the United States, Europe, and Australia. Genetest is a publicly-funded medical genetics resource developed for physicians, other healthcare providers, and researchers. Their Web site provides information on genetic testing and its use in diagnosis, management, and genetic counseling. In all, we obtained test information from 95 laboratories. Table 12 in Appendix B presents a list of all laboratories from which test information was obtained.[1]

Once an initial list of tests and laboratories offering each test was compiled, we searched for laboratory-specific information through the laboratory details page located in the AMP test directory. The laboratory details page of the AMP Web site provides a list of all the molecular tests performed by each laboratory along with the information on whether any of the tests were developed by the particular laboratory itself or performed by the laboratory using a "commercial kit." Unfortunately, the laboratories often do not indicate whether they use a FDA-approved or cleared commercial kit (a full testing system), FDA-cleared analyte specific reagents (ASRs, also called "commercial kits" by some laboratories), and, in some cases, the source of the commercial kit.[2] Information about commercial kits was also not often available from individual laboratory Web sites. Thus, we conservatively included all tests using "commercial kits"—which could be

[1] All laboratories located outside of the United States were excluded from our list.

[2] A listing of all FDA-approved test kits as of December 11, 2009, is provided in Table 24 and Table 28.

either full testing systems or laboratory-developed systems using commercial ASRs—in our inventory to decrease the risk of missing significant LDMTs. In our inventory, these tests are tabled separately from confirmed LDMTs.

Results

Using information from the AMP Web site, the laboratory details page, and individual laboratory Web sites, we created a set of tables that list laboratory-developed molecular tests available as of October 31, 2008, that are relevant to the Medicare over-65 population. The tables are located in Appendix B of this report. Separate tables were created for the following clinical conditions: infectious diseases, solid tumors, and hematopathology. For infectious diseases, separate tables were created for diseases caused by bacterial agents (Table 14 and Table 19), viral agents (Table 15 and Table 20), or fungal or parasitic agents (Table 16). For all clinical conditions, separate tables were created for tests clearly identified as laboratory developed (Table 14 through Table 18), and those available through commercial ASRs or kits (Table 19 through Table 23). We include tables of tests available through "commercial kits" because, as indicated previously, it was unclear from the information provided in the AMP test directory whether the "commercial kits" used by laboratories are FDA-approved full testing systems or ASRs. When specified in the AMP test directory or on individual laboratory Web sites, we indicate the source of the commercial full testing system.

Further information about how the tables are organized is presented in the *Guide to Molecular Test Tables* section of this report, which is located in Appendix B.

In all, we cataloged 1,441 molecular tests, of which 812 were clearly identified as laboratory-developed tests and 629 were tests that used commercially available full testing systems or ASRs (meaning that some of them are still laboratory developed, if they use commercial ASRs). Table 1 below summarizes the results of our cataloging efforts. The test numbers reported in the table were calculated by ECRI Institute by summing the number of laboratories performing a test for a specific clinical condition. If two laboratories both offer a test developed in-house for the detection of the same clinical condition (e.g., mycobacterium tuberculosis), we counted them as two separate LDMTs. We counted each laboratory separately because testing protocols may vary significantly across the laboratories even though the tests are intended to detect the same pathogen/clinical condition.

Table 1. Summary of Molecular Tests

Test Category	Number of LDMTs	Number of Tests Using Commercial Kits or ASRs	Total Number of Tests
Infectious Disease Tests, Bacterial	153	151	304
Infectious Disease Tests, Viral	259	214	473
Infectious Disease Tests, Parasitic or Fungal	34	0	34
Solid Tumor Tests	145	97	242
Hematopathology Tests	221	167	388

Overall, we cataloged 811 molecular tests for infectious diseases. The majority of these tests used PCR (including PCR-based methods and PCR-like nucleic acid amplification techniques) as the testing method, and were used to confirm diagnosis. Of these 811 tests, 304 tests were used for bacterial infectious diseases that covered a wide range of conditions, including clostridium difficile, legionella pneumophilia, and mycobacterium tuberculosis. Most tests using commercial kits or commercially available ASRs were for tuberculosis (n = 29), and in most cases, Gen-Probe was the laboratory that supplied the test kit (n = 24). No one condition appeared more prevalent among the laboratory-developed tests.

Tests used for viral infectious diseases also covered a number of conditions, ranging from cytomegalovirus to West Nile virus. The majority of tests, both in-house and commercially available, were for the following conditions: hepatitis (in-house = 20, commercial = 67), herpes simplex virus (in-house = 63, commercial = 5), and human immunodeficiency virus (in-house = 9, commercial = 77). Finally, tests used for parasitic- or fungal-related infectious diseases covered a number of conditions, ranging from blastomyces dermatitidis to toxoplasma gondii.

Overall, we cataloged 630 molecular tests currently available for clinical use in oncology, including solid tumors and hematological malignancies. Most of these tests are for the purpose of diagnosis; only limited numbers of tests are for disease monitoring or for determining treatment strategies. The most commonly used techniques in these tests are PCR and FISH. Breast cancer, colorectal cancer, sarcomas, acute myeloid leukemia, and B and T cell neoplasms are the conditions that have the highest number of tests (or laboratories offering the tests) available.

Chapter 2. How Is Analytic Validity Established for Laboratory-Developed Molecular Tests?

For this Key Question, we were asked to look at how analytic validity is established for laboratory-developed molecular tests. Specifically, we were asked to cover the following aspects of analytic validity:

- Accuracy
    - In comparison to reference methods
    - Type of samples tested (i.e., control material, patient samples, etc.)
    - Number of samples tested
    - Methods for setting a cut-off
- Precision/reproducibility
    - Repeatability: replication studies on a single specimen using a single molecular test method and the same equipment
    - Reproducibility: replication studies assessing day-to-day, operator-to-operator precision. If multiple instruments are used, instrument-to-instrument reproducibility should be assessed; if the test is performed in multiple laboratories, site-to-site reproducibility should be also assessed.
    - Duration of study
    - Type of sample tested (i.e., control material, patient samples, etc.)
    - Number of samples tested
    - Performance near clinically critical cut-points and covering the assay's detection range
- Assay linearity, recovery and high-dose hook-effect
- Limit of detection and limit of quantitation (at low and high ranges)
- Analytical specificity (cross-reactivity, interference)
- Matrix effects
- Quality assurance for manufactured and/or purchased reagents utilized in laboratory-developed molecular tests
- Pre-analytical factors influencing assay results
- Assay output (reading ranges)

Methods

To address Key Question 2, we provide an overview of the processes used to establish the analytic validity for new laboratory-developed tests. In the literature search, systematic reviews relevant to the questions were given highest priority, followed by other publication types, including evaluation studies. Recent narrative reviews were utilized as necessary to address questions not covered by systematic reviews or primary studies. Meeting abstracts, Web-based publications, and other "gray literature" were included if published literature was not available to address the various sub-questions. The literature search strategy for this Key Question is provided in Appendix A of the report.

In addition to the information from the literature, expert input was also utilized in addressing Key Question 2. We also selected a representative sample of molecular tests and discussed the validation process used for each test.

Results

The initial step of developing a molecular test is to design and optimize the test. For example, a large number of tumor samples may be screened to identify a panel of markers that appear to predict response to chemotherapy. The initial developmental stage is not discussed here. Once the test has been developed, it needs to be validated. The College of American Pathologists (CAP) published recommendations on how to perform analytic and clinical validation studies and the checklists used for CAP accreditation describe standards to be met in validation studies.[7] The American College of Medical Genetics (ACMG) guidelines on molecular testing state that each laboratory must validate the analytical performance characteristics (sensitivity, specificity, reproducibility) of the technique chosen for analysis of each gene.[8] New York State Clinical Laboratory Standards of Practice guidelines on validation also state that laboratories must establish performance specifications for accuracy, precision, reportable range, reference intervals, analytical sensitivity and specificity, and other applicable performance characteristics (www.wadsworth.org/labcert/TestApproval/submitguide.htm). Validation studies must be submitted to New York State and approved before the test may be commercially offered for use on samples submitted from New York State.[9] The Clinical Laboratory Improvement Amendments (CLIA) program requires that any new test be validated by the laboratory, but unlike New York State, does not require submission of a formal demonstration of validation prior to offering the test, as long as the laboratory is in compliance with CLIA regulations.[10]

The following sections of this chapter address specific aspects of the process for validating analytical performance of molecular tests based on the literature described in the *Methods* section. In addition, the Clinical and Laboratory Standard Institute (CLSI), a well-regarded organization that develops laboratory standards based on voluntary consensus, has published a series of molecular-test-related guidelines (listed in Chapter 8 and Appendix D). Some of these CLSI guidelines discuss analytic validity regarding microarrays[11] (pp. 61-63), nucleic acid amplification for hematopathology[12] (p. 34), molecular diagnostic methods for genetic diseases[13] (pp. 38-44), florescence in situ hybridization[14] (pp. 11-20), and multiplex nucleic acid assays[15] (pp. 35-42). Refer to the CLSI guidelines for the detailed discussions. Another useful source with

detailed descriptions of the processes involved in establishing analytic validity of microbiology tests is the review by Wolk, Mitchell and Patel (2001).[16]

Clinically oriented literature on validation of diagnostic tests sometimes merges the concepts of analytic validity (at the level of the substance being detected or measured) with those of clinical validity (at the level of the disease or condition), particularly when referring to test "accuracy." Most evidence-based processes for evaluating diagnostic tests assume that evaluating clinical validity will address any analytic validity problems, and thus do not formally consider analytic validity. However, the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group states that formal evaluation of analytic validity for genetic technologies is important because the technologies are complex and rapidly evolving and tests may not have been fully validated in multiple sites, for all populations of interest, or under routine clinical laboratory conditions over time; in addition, review of analytic validity can also determine whether clinical validity can be improved by addressing test performance.[17]

Strictly speaking, analytic validity includes the concepts of accuracy, precision, analytic sensitivity, analytic specificity, linear range and determination of normal range or cut-off points for positivity. The CLIA regulations require laboratories to establish these parameters prior to offering the test to the public (42 CFR 493.1253). When, as is the case with many molecular tests, there is no reference standard for the analyte, analytic validity is more narrowly defined, and descriptions of "accuracy" are actually about "diagnostic accuracy," or "clinical validity."

Accuracy

Accuracy in Comparison to Reference Methods

Accuracy refers to how well the test measures what it purports to measure, and is determined for most laboratory tests by using the test to detect and often to measure the quantity of a known substance of a known concentration in a specimen. The identification of true-positive samples and the amount of the substance present in the sample are determined independently by a reference method.

Establishing reference methods for molecular tests is problematic. The analytical accuracy of many quantitative molecular assays cannot be established because no reference standard exists. An additional complication is that in many cases, the PCR-based assay being validated may be more sensitive than the current "gold standard" reference assay.[18-21] Direct sequencing of genetic material can be used as the "gold standard" for many tests intended to detect mutations.[21-23] Other methods that can be used to confirm the accuracy of molecular tests include specific hybridization, nested PCR, or restriction enzyme digest assays.[21] Restriction enzymes are used to break DNA at specific sequence sites, creating more specific DNA fragments. These fragments can then be hybridized, tagged, and separated by electrophoresis, creating patterns indicative of mutations. Nested PCR uses two sets of PCR primers for a single target. The first set of primers amplify the target as with any standard PCR. The second set of primers (nested primers) bind within the product from the first PCR amplification and produce a second PCR product that will be shorter than the first one. Nested PCR is used to reduce the chance of amplifying unwanted DNA sequences. The ACMG guidelines indicate that in the absence of "gold standards" for comparison of results of new assays, the splitting of samples with another laboratory with an established clinical assay may be considered.[8]

The American Society of Clinical Oncology, in conjunction with the College of American Pathologists, suggests comparing the new assay to a previously validated assay, and if the results are 95% concordant, the new assay may also be considered to be valid.[24] One laboratory published the results of its attempts to validate their in-house PCR assay by following this approach.[25] The laboratory compared the results of its *HER2*/*neu* (also known as *ERBB2*) assay to four other available methods of measuring *HER-2*/*neu* amplification or over-expression. Complete agreement between methods was obtained for only 94.5% of the 163 samples tested. Because no true reference standard was available, the discrepancies could not be resolved.

Type of Samples Tested

The question of how and from where specimens are to be obtained for validation of a molecular test has not been clearly addressed in the literature. For some conditions, such as rare genetic disorders, there are very few positive clinical samples available.[20,22] There are some public repositories of cell lines and other biological materials that may be suitable for validation of some molecular tests, for example, both the Coriell Cell Repositories (http://ccr.coriell.org) and the American Type Culture Collection (http://www.atcc.org) maintain an extensive collection of biological materials. It is, however, important to ensure that the specimens used to validate the test are collected, stored, and processed in the same way samples will be prepared for actual clinical use of the test, and therefore artificially constructed samples may not be appropriate for validating many molecular tests.[16,20] In its 2008 report on the U.S. system of oversight of genetic testing, SACGHS recommended that the Human Health Services (HHS) should ensure funding for the development and characterization of reference materials, methods, and samples for the validation of genetic tests.[3]

Number of Samples Tested

Dimech et al. recommend that at least 100 positive and 100 negative samples be tested during the analytical validation phase.[21] Dimech et al. further suggest that if positive samples are difficult to obtain, a minimum of 20 "real" positive samples should be tested, and this data should be supplemented with tests of artificially-constructed positive samples.[21] The American Society of Clinical Oncology/College of American Pathologists suggests testing 25 to 100 samples during the analytical validation phase.[24] Prence recommends testing at least 20, preferably more than 100, samples to establish validity, noting that care should be taken in selecting samples in order to simulate a clinically relevant population.[26] Factors such as age, sex, pregnancy, ethnic background, use of medications, and anything else that could affect the test result should be considered in determining an appropriate sample number. Another factor that needs to be considered is the use of healthy normal volunteers as negative samples, because such practice may skew the testing results. In some cases, negative samples selected from patients with other, similar, disorders may be necessary to truly validate the test.

Methods for Setting a Cut-off Threshold

The cut-off threshold is the point at which the test is declared "positive" or "negative." Methods to establish the cut-off point depend on the test and its intended purpose. For example, a test intended to measure levels of virus in the blood may have no cut-off point; whereas a test intended to detect amplification of *HER2*/*neu* (i.e., *ERBB2*) may be declared "positive" if the test indicates three or more copies of the gene are present.

Precision/Reproducibility

Repeatability/Precision

Repeatability is defined as replication of results when the assay is performed multiple times on a single specimen. Repeatability is also referred to as precision when the test result is expressed quantitatively.[16] Both Dimech et al. and Prence recommend testing a dilution series, with each sample tested at least 20 times, to establish the precision of the assay.[21,26] The degree of precision is commonly expressed in terms of the coefficient of variation (CV) of the test.

Nygarrd et al. have reported that the repeatability of PCR amplification from small-size samples is limited.[27] Although samples with small amounts of starting mRNA can reproducibly be amplified, when the sample size (defined as number of cells) falls below certain levels, the proportion of transcript in the original material is not reproducibly maintained in the amplified material. For example, in samples of 1000 cells, only transcripts expressed with at least 121 transcripts per cell were reliably amplified in proportions representative of the starting material, and for samples of 250 cells, only transcripts expressed at 1806 or more copies per cell reliably amplified in proportions representative of the starting material. These results have important implications for test assays that rely on microdissected cells or other extremely small samples.[27]

Reproducibility

Reproducibility refers to replication studies assessing day-to-day and operator-to-operator precision. If multiple instruments are used, instrument-to-instrument reproducibility should be assessed; if the test is performed in multiple laboratories, site-to-site reproducibility should also be assessed.[9] We identified one study that discussed how reproducibility of molecular test assays should be assessed, the details of which are described below.[21]

Type of Sample Tested

Reproducibility studies may be performed on the same dilution series used to establish the precision of the test.[21] However, if the assay incorporates an extraction step, reproducibility of the extraction step should be incorporated into the validation studies, and likewise for any other steps of the procedure.

Number of Samples Tested

Dimech et al. recommend performing reproducibility testing at clinically critical cut-points covering the assay's measurement range at no more than ten times this level.[21]

Performance Near Clinically Critical Cut-points and Covering the Assay's Detection Range

Reproducibility studies of assays should provide an estimate of the precision of the method at analyte concentrations near the cut-off threshold and at clinically critical cut-points covering the assay's measurement range.[21]

Assay Linearity, Recovery, and High-dose Hook Effect

Assay linearity is defined by the Clinical Laboratory Standards Institute (CLSI) as "the ability (within a given range) to provide results that are directly proportional to the concentration (amount) of the analyte in the test sample."[28] Linearity of tests is established by testing a dilution series of a positive sample.[21] The term "recovery" refers to "the measurable increase in analyte concentration or activity in a sample after adding a known amount of that analyte to the sample."[28] For some quantitative tests to give accurate results, there must be an excess of reagents, relative to the analyte being detected. As the concentration of analyte begins to exceed

the amount of reagent, the dose response curve will plateau, and with further increase may paradoxically become negatively sloped in a phenomenon termed "High-Dose Hook Effect." Failure to validate the potential for high-dose hook effect (by linear dilution of samples) can result in severe underestimation of the true amount of the analyte.

Analytic Sensitivity or Lower Limit of Detection/Quantitation

Analytical sensitivity describes how effectively a test can detect all true positive specimens. Alternatively, for quantitative tests, analytic sensitivity (also referred to as the lower limit of detection), may be defined as the smallest quantity of a substance that can be reliably detected or quantified. For example, for a molecular test the lower limit of detection could refer to the lowest number of organisms or RNA copies that can be reliably and reproducibly detected by the assay. This parameter is established by serially diluting samples and running the assay repeatedly.[20] The World Health Organization (WHO) has established that limits of detection for certain targets should be expressed as IU/ml.[16] Standard reference materials to establish an absolute measure of limit of detection for molecular tests are currently limited to a few viruses (hepatitis B and HIV) and a genetic test for the Factor V Leiden mutation, which causes a coagulation disorder.[29,30]

Analytical Specificity

Analytical specificity refers to the ability of a test to measure the target substance when potentially interfering or cross-reacting substances are present in the specimen. For example, with many standard chemistry tests, the presence of hemolysis or bilirubin in the sample would cause interference with measurement of the desired substance. Potential for cross-reactivity is often not fully investigated during validation of molecular tests for practical reasons. For example, a real-time PCR assay intended to amplify DNA from a particular strain of bacteria cross-reacts with and also amplifies DNA from a different strain of bacteria. In order to detect this cross-reaction the developers of the test would have to first suspect it may occur and then somehow obtain suitable samples to verify the effect. Alternatively, the cross-reaction may be detected accidently during the validation process. However, careful design of primers can reduce the potential for cross reactivity. The chosen sequence should be compared to genome sequences in appropriate databases to confirm the specificity of the primer.[21] Dimech et al. suggest creating a sample spiked with a high concentration of potentially cross-reacting organisms and human cells to test for analytical specificity.[21]

Matrix Effects

The term "matrix effects" refers to the combined effect of all components of the sample other than the analyte on the detection or value of the measurement of the analyte. The potential for matrix effects is often a concern when using external controls that differ from fresh specimens typically used in a clinical laboratory. CLSI distinguishes this concept from "interference," which implies an identified substance or property (e.g., pH, surface tension, viscosity) of the patient sample, control sample or calibration material that alters the test result.[31] In the context of molecular testing, the most commonly encountered matrix effect is the presence of substances in the sample that inhibit PCR reactions. Such substances are commonly encountered and may cause false-negative results or under-estimation of the amount of the target substance in the sample. However, inclusion of an endogenous or spiked exogenous control in the test methodology can sometimes detect or control for this possibility.[19,20]

An internal control can be constructed that contains nucleic acid of a different size and sequence than the target, but with the same target primer sequences at each end; once constructed, the control can be added to each sample before beginning the assay. Alternatively, a housekeeping gene (a gene expressed in virtually all cell types) such as β-globin can be simultaneously amplified along with the target sequence.[20] Ambion manufactures ready-to-use RNA products (Armored RNA) that can be used as controls in many types of assays.[20]

Other possible causes of matrix effects include cross-contamination during sample processing, inclusion of normal, non-diseased tissue with the diseased tissue of interest (e.g., normal tissue contained within a tumor block), and tissue from a source additional to the desired sample (e.g., blood cells in a biopsy specimen). Sources of cross-contamination should be assessed and preventive measures taken accordingly.[32]

Quality Assurance for Commercial Reagents

Manufacturers of both analyte-specific reagents and general laboratory reagents are required by FDA to follow current Good Manufacturing Practices.[33] Forbes recommends that laboratories ensure the quality of PCR test reagents by performing functional validation assays to determine the efficacy of new reagents run in parallel with old reagents.[20] Most "good laboratory practice" guidelines concur with this suggestion.[9,34,35]

Pre-analytical Factors Influencing Assay Results

Pre-analytical factors that can influence assay results include type of specimens (e.g., fixed tissue vs. fresh-frozen tissue), how specimens are collected, proper processing and storage to prevent contamination or degradation of the sample, and transport of the sample. The methods used to extract nucleic acid from the samples, if performed, can also influence the results.

Assay Output

Assay output is the raw data generated from a molecular test procedure. Numerous options exist for presenting the output of molecular tests, but the data require further clinical interpretation in conjunction with controls and patient information before clinical laboratory reports can be issued.[36]

Systematic Reviews Addressing Analytic Validity

Literature searches identified two systematic reviews addressing analytic validity. As part of a technology assessment prepared for AHRQ in 2006, Matcher et al. addressed the analytic validity of the tests for detection of cytochrome p450 polymorphisms in adults with depression.[37] They stated that the "gold standard" reference for these tests is bidirectional sequencing. In this method, forward and reverse primers are utilized, permitting confirmation of the sequence in the region of the mutation. They identified 12 published articles and two documents from the FDA Web site (on performance of the Roche AmpliChip®) that described methods for genotyping various *CYP450* enzymes. Only four of the studies used the "gold standard" reference of DNA sequencing; the others compared their results to other methods of genotyping, or to published allele frequencies in populations similar to the ones employed in the study. Sensitivity and specificity were generally high (in the range of 94 to 100 percent) for the various tests. Sample sizes used in the validation studies ranged from approximately 50 to approximately 400, of which most were negative for any of the target polymorphisms; the numbers of positive samples were generally very low, in the single digits for most of the tests and polymorphisms. Some of

the validation studies also reported on the reproducibility and repeatability of the tests. Repeatability assays varied, and were performed on one to four samples anywhere from only twice to up to 12 times. Reproducibility assays also varied, and may have incorporated between-laboratory, between-operator, and day-to-day assays; however, few studies reported performing all three types of reproducibility assays.

Another systematic review performed by the Johns Hopkins EPC under contract to AHRQ was released in 2008. In this review, the authors examined analytic and clinical validity of three currently marketed molecular tests for risk stratification of patients with breast cancer. They found limited published evidence for analytic validity, with the most complete documentation being for the Onco*type* DX® assay (Genomic Health, Inc., Redwood City, CA). The authors noted that, given the absence of reference standards, the majority of published analytical validation studies only addressed repeatability and reproducibility rather than the other test properties discussed above.[38] Unpublished validation data may be available in the testing laboratory.

Establishment of Analytic Validity for Molecular Tests - Examples

Our searches revealed that few validation studies of laboratory-developed or commercially available molecular tests have been published. We selected a few tests representing the following categories relevant to the Medicare population:

- tests used for diagnostic purposes in symptomatic individuals,
- tests used as prognostic indicators,
- tests used to monitor response to therapy, and
- tests used to choose therapies for a known disease entity or used to adjust medication dosing.

Tests Used for Diagnosis in Symptomatic Individuals: Parkinson's Disease

Some commercial laboratories, such as Athena Diagnostics,[39] offer genetic tests to aid in diagnosis of Parkinson's disease (PD). We were unable to identify any published validation studies of these specific laboratory-developed tests. However, the Wadsworth Center published a clinical and analytical validation study of a genetic test to aid diagnosis of Parkinson's disease (PD) in 2006.[40] This validation study is typical for molecular tests used for diagnosis.

PD is a progressive neurodegenerative disorder. Differential diagnosis of PD can be complicated, especially in the early stages of the disease. At present, a definitive diagnosis can only be achieved after death by examination of the brain. PD is caused by both genetic and environmental factors. A number of mutations of different genes have been linked to PD. Mutations in the gene *LRRK2* are associated with the most common forms of PD, and the most commonly identified mutation in *LRRK2* is *G2019S*.

The validation study published by the Wadsworth Center recruited 1518 individuals diagnosed with PD and 1733 without PD (a case-control study). The test being validated uses genomic DNA purified from peripheral blood and employs a real-time PCR (Taqman) assay. All identified mutations were verified by direct sequencing, and results from a random sample of 26% of the subjects were also verified by direct sequencing. The sequencing verified that the Taqman assay had 100% sensitivity and specificity in detecting the mutation. The sensitivity of

the assay in diagnosing PD was poor, only 1.3%, but the specificity was very high at 99.9%. The authors concluded that the test may have clinical utility for confirming the diagnosis of PD in a subset of suspected cases. They further speculated that the detection of the mutation in asymptomatic carriers will predict a substantially increased risk of PD, but does not guarantee that the individual will develop PD nor can it predict age at onset.

As discussed previously, case-control studies tend to over-estimate the accuracy of diagnostic tests. The case-control design of this study weakens its claim to have established the clinical validity of this test (further discussion of clinical validity and utility of molecular testing is in the next chapter). Verification of mutation identification by sequencing does establish the analytical accuracy of the test. However, other aspects of analytic validity, such as reproducibility and repeatability, were not addressed in the published report.

Tests Used for Diagnosis and Monitoring: Heptimax®

Heptimax® is a laboratory-developed test developed by Quest Diagnostics. The test is used to confirm active hepatitis C viral (HCV) infection, to monitor response to HCV therapy, and to confirm resolution of infection.[41] The test uses real-time PCR to measure viral load in plasma.

HCV infection is a major cause of hepatic disease. Acute HCV infection is often asymptomatic and often is not diagnosed. Most patients then go on to develop chronic HCV infection, which is associated with increased risk of cirrhosis of the liver and hepatocellular carcinoma.

Quest Diagnostics has not published a validation study of Heptimax in the peer-reviewed medical literature. Unpublished data may be available in the testing laboratory. The company reports the range of the assay as 5 to 50,000,000 IU/ml on its Web site.[42] The Web site references seven publications as supporting the clinical utility of the test, but none of the published studies appear to have actually used or studied Heptimax® specifically.[42]

Tests Used to Determine Prognosis and Guide Choice of Therapy: Onco*type* DX®

The Onco*type* DX® assay is a gene-profiling assay that uses real-time PCR to measure the expression levels of a panel of 21 genes in breast tumor tissue samples. Physicians use a sample kit provided by Genomic Health to send fixed, paraffin-embedded breast tumor tissue to the Genomic Health laboratory. Within two weeks of specimen receipt, the laboratory reports a "recurrence score" of 0 to 100 and an explanation of the estimated risk of tumor recurrence associated with that score. The assay results are intended to be used in conjunction with other clinical information to guide treatment decisions. Although the Onco*type* DX® assay may fall into the in vitro multivariate index assay (IVDMIA) class that the FDA has recently declared interest in regulating, it has been marketed since 2004 as a laboratory-developed test, and its publication history is instructive. One of the systematic reviews of clinical validity that we identified (Lyman et al.[43]) reviewed the Onco*type* DX® assay and other similar assays intended to predict the likelihood of breast cancer recurrence. Clinical validity is addressed in detail in the next chapter.

On the company's Web site, Genomic Health lists five published studies that were used to develop the Onco*type* DX® assay.[44] After development of the assay, the company published one analytical validation study and two clinical validation studies. The analytical validation study was performed on a pooled sample of fixed tissues. The study reported data on the reproducibility (across instruments, operators, reagents, and day-to-day variation) and the

repeatability (multiple assays run on the same sample) of the assay, and the linear performance of the assay over a broad range of sample concentrations.[45] The authors of the study reported that analytical accuracy could not be assessed because there is no standard reference material for each of the 21 analytes. They did measure quantitative bias by comparing predicted RNA concentrations for each gene to expected RNA concentrations calculated for a range of sample dilutions.

Tests Used to Adjust Medication Dosing: Genetic Testing to Guide Warfarin Dosing

Warfarin is an oral anticoagulant prescribed to treat a variety of health conditions. Warfarin acts by interfering with the synthesis of clotting factors in the liver. Bleeding is a common adverse event associated with taking warfarin, and establishing the safe and effective dose of warfarin for each patient can be difficult. Certain polymorphisms in the genes *CYP2C9* (which encodes the protein cytochrome *P450 2C9*) and *VKORC1* (which encodes vitamin K epoxide reductase complex subunit 1) affect the metabolism and action of warfarin. In August 2007, the FDA updated the product label for warfarin (Coumadin®) to include genetic variations in *CYP2C9* and *VKORC1* as factors to consider for more precise initial dosing.[46]

At least three laboratories offer laboratory-developed tests for genetic variations in *CYP2C9* and *VKORC*1: Genelex's Warfarin Target Dose Safety Test,[47] Clinical Data Inc.'s PGxPredict: Warfarin™,[48] and Kimball Genetics Inc.'s Warfarin Dose Advise™.[49] However, we were unable to identify any published studies validating these tests, and the company Web sites contained no information about their development or validation.

A number of kits to assess genetic variation in *CYP2C9* and/or *VKORC1* have received clearance for marketing from the FDA under the 510(k) process (refer to Table 26 in Appendix B). For example, the Verigenen Warfarin Metabolism Nucleic Acid Test (Nanosphere, Inc., Northbrook, IL) was cleared in September 2007.[50] The company reported information about their test's accuracy relative to bi-directional sequencing, reproducibility, limit of detection, and lack of interference by five common contaminants to the FDA as part of their 510(k) application.

Roche Molecular Systems, Inc. received 510(K) clearance of a test to evaluate genetic variation of *CYP2C19* and *CYP2D6* in December 2004 (Roche Amplichip CYP450).[51] Validation studies of the Amplichip for use in guiding medication dosing (medications for depression) were evaluated in the Matcher et al. systematic review, discussed above.[37]

Chapter 3. What Processes Have Been Developed for Examining Clinical Validity and Clinical Utility of Molecular Tests?

This chapter of the report addresses Key Question 3, which focuses on existing processes for examining the clinical validity and clinical utility of molecular tests. Particularly, we were asked to examine the features unique to molecular testing in the following areas:

- Clinical validity: Test characteristics (sensitivity, specificity, predictive values, likelihood ratios)
- Clinical utility: whether the results of the test can be used to pursue effective treatment or provide other concrete clinical benefit

Methods

To address this Key Question, we first provide an overview of the processes used to establish the clinical validity and utility of a molecular test. We further discuss the unique challenges in assessing clinical validity and utility of molecular tests. The overview and the discussion are based on the literature we considered relevant to the topic; both reviews and clinical studies are included in this discussion.

To examine the clinical test properties of molecular tests, we also consulted systematic reviews that evaluated clinical validity and/or clinical utility of various molecular tests. For clinical validity, we examined systematic reviews that reported on test characteristics, such as sensitivity, specificity, predictive values, and likelihood ratios. For clinical utility, we considered reviews that reported on patient-oriented health outcomes, such as survival, recurrence of disease, and treatment changes. The literature search strategies for this Key Question are provided in Appendix A of the report.

Quality of the systematic reviews was assessed with the 'assessment of multiple systematic reviews' (AMSTAR) measurement tool (Table 29).[52] The AMSTAR consists of 11 items, which have been tested for face and content validity. The items assess whether or not a systematic review includes important elements, such as a comprehensive literature search, assessment of study quality, appropriate methods to combine study findings, and assessment of publication bias. Responses to each item are checked as 'Yes' if the review includes that item, 'No' if it does not, 'CA' if the item cannot be answered by the information provided in the review, or 'NA' if the item is not applicable. The AMSTAR does not provide a method for rating the quality of a review. To rate the quality of the reviews, we applied the following criteria: a rating of 'High' if the review received mostly 'yes' responses (at least 8), a rating of 'Low' if the review received mostly 'no' responses, and a rating of 'Moderate' if the review received mixed responses.

Results

Assessing Clinical Validity of Molecular Tests

The clinical validity of a molecular test refers to the test's ability to detect or exclude a disease or a condition in patients compared with a criterion standard or reference test. The methods used to evaluate the clinical validity of molecular tests are similar to those used for any other diagnostic test, which include measuring the following test characteristics: sensitivity, specificity, predictive values, and likelihood ratios.

Sensitivity is the probability of a positive test result when disease is present. Specificity, on the other hand, is the probability of a negative test result when disease is absent. The acceptable levels for clinical sensitivity and specificity may vary depending on the purpose for which a test is used. While sensitivity and specificity are the most widely used outcome measures, they are sensitive to spectrum bias. Spectrum bias may occur when the study population has a different clinical spectrum (more advanced cases, for instance) than the population in whom the test is to be applied.[53]

Other commonly used measures of diagnostic test performance are the positive and negative predictive values, which reflect the perspective of a clinician interpreting a given test result. The positive predictive value (PPV) of a test is the probability of an individual actually having the disease/condition when the test result is positive. The negative predictive value (NPV) is the probability of an individual not having the disease/condition when the test result is negative. Unlike sensitivity and specificity, predictive values are influenced by the prevalence of disease in the population of individuals being tested. For example, in a situation where disease prevalence is very low, say 1%, the negative predictive value of most tests will easily exceed 95%, given that 99% of the population does not have the disease.

Two other measures of diagnostic test performance can be calculated that may be more clinically useful: the positive likelihood ratio and the negative likelihood ratio.[54,55] The positive likelihood ratio measures the ability of the test to accurately "rule in" disease, whereas the negative likelihood ratio measures the ability of the test to accurately "rule out" disease. Likelihood ratios can be directly used in Bayes' theorem to calculate posttest odds of having disease from the pretest suspicion of the individual's odds of having disease.

Two elements are particularly important to consider when assessing the clinical validity of genetic tests—penetrance and modifiers. Penetrance is a measurement of the proportion of individuals in a population with a disease-related genotype or mutation who develop the disease. It is expressed numerically. For example, if 100 individuals all have a particular gene mutation but only 80 of them have the condition associated with that mutation, then the mutation is said to be 80% penetrant.[3] Modifiers include other genetic or environmental factors that may interact with the genetic alteration being studied and the outcome(s) of interest. Modifiers can affect expressivity, which refers to the variability of signs or symptoms of disease among individuals with the same genotype.

One frequent tactic for establishing molecular test validity is the use of the case-control study. Case-control studies are studies in which a collection of "cases" (known to have the condition or mutation of interest) and a collection of "controls" (known not to have the condition or mutation of interest) are assembled and tested with the experimental diagnostic test. Case-control studies are generally considered to be a poor method of evaluating diagnostic test

accuracy because they have been shown to over-estimate test accuracy. This occurs because the studies usually include only the unambiguous cases, those that are clearly "positive" and those that are clearly "negative," and fail to include the difficult-to-diagnose cases. Pai et al. reported that this drawback of the case-control design applies to molecular tests.[56,57] The most rigorous study design for assessing the clinical validity of a molecular test, as with any other diagnostic test, is a prospective blinded comparison of the test and a reference standard in a consecutive series of patients from a relevant clinical population.

The Onco*type* DX™ assay (Genomic Health, Inc., Redwood City, CA), described in Chapter 2, is used to assess prognosis and guide choice of adjuvant therapy in breast cancer patients (hormonal therapy alone versus hormonal therapy plus chemotherapy). As mentioned in the previous chapter, published studies on the development, clinical validation, and clinical utility of the Onco*type* DX™ assay are listed on the Genomic Health Web site. The National Cancer Institute (NCI) is sponsoring an on-going clinical trial that began in 2006 to evaluate the effect of adjuvant chemotherapy on disease-free survival in women with "Mid-Range" Onco*type* DX™ Recurrence Scores®. The study (Trial Assigning Individualized Options for Treatment/TAILORx) is planned to enroll about 10,000 breast cancer patients and assess recurrence and mortality outcomes for 20 years. The principal objectives of the trial are:

- To determine whether hormonal therapy alone is equivalent to hormonal therapy plus chemotherapy in women whose tumors meet established clinical guidelines for adjuvant chemotherapy and whose Onco*type* DX™ Recurrence Score® test results are in the "uncertain chemotherapy benefit" category as set by study investigators (Recurrence Score® results from 11 to 25).
- To create a tissue and specimen bank for patients enrolled in the trial, including formalin-fixed, paraffin-embedded tumor specimens, tissue microarrays, plasma, and DNA obtained from peripheral blood.

Challenges in Assessing Clinical Validity of Molecular Tests

The clinical validity of a molecular test is influenced by a number of factors, including the adequacy of information available to determine how accurate the test is in detecting a health condition, the purpose of the test, and the prevalence of the disease or condition for which the test is being conducted. Forbes noted several issues associated with validating the clinical sensitivity and specificity of an amplification test (e.g., PCR) on clinical specimens for infectious diseases.[20] The most significant is the selection of the "gold standard," whose results will be compared to those obtained with the amplification test. Culture has been traditionally used as the final arbitrator in determining whether a clinical specimen is truly positive or not for a given pathogen. However, according to Forbes, numerous incidents during the past decade have arisen that have demonstrated that culture may be an imperfect standard. She gives two examples in which PCR has been shown to be more sensitive than culture—the detection of *Bordetella pertussis* in respiratory samples and herpes simplex virus in cerebrospinal fluid. Recognizing that the gold standard may be imperfect, some laboratories use "discrepant resolution" in which patients for whom the reference standard and test method disagree are subjected to a "third" resolver test. A number of objections, however, have been raised with regard to using this method. For instance, Marr and Leisenring argue that discrepant resolution methods are generally more expensive and invasive and are typically biased in favor of amplification tests.[58]

They suggest resolving discrepancies either through repeated testing or by using, when possible, multiple imperfect reference standards.

A somewhat analogous issue to selecting a "gold standard" in assessing the clinical validity of a molecular genetic test is the characterization of the mutations being tested. In order to accurately validate a genetic test, the exact characterization of the mutations being tested is needed. However, according to Zimmern and Kroese, in many instances, all the main causative mutations will not be known and this will reduce the sensitivity and, hence, clinical validity of the test.[59] Further, the key causative mutations for a particular disorder may vary among different populations. Zimmern and Kroese highlight the studies of clinical sensitivity of the ACMG panel of 25 mutations for cystic fibrosis. The overall results of the studies estimated that the clinical sensitivity of the panel was 71.9% for non-Hispanic Caucasians, 41.6% for African Americans and only 23.4% for Asian Americans. The clinical sensitivity of this test, according to Zimmern and Kroese, was limited by the mutations chosen to be included in the panel, which highlights the importance of knowledge of the frequency of specific genetic variations in a defined population.

A number of other issues, such as heterogeneity of disease, mosaicism, and prevalence of disease, are important to consider when assessing the clinical validity of molecular tests. Heterogeneity of disease is particularly relevant to genetic tests, because a particular genetic condition may be caused by more than one gene, or by more than one variant within the gene.[60] Thus, a genetic test will perform poorly if genes or variants other than the one tested for are responsible for the disease (i.e., locus heterogeneity or allelic heterogeneity).

False-negative results in individuals may also occur in genetic tests when there is mosaicism, where only a proportion of cells contain a mutation.[60] This occurs in genetic conditions caused by sporadic mutations, for example, tuberous sclerosis complex. In this case, false-negative results may occur because of the small number of cells containing the mutation in the sample being tested. Finally, assessing clinical validity may be particularly challenging in the case of tests for ultra-rare diseases.[3] As relatively few people have these diseases, gathering statistically significant data can be extremely challenging. Thus, prevalence is a factor in determining how much data on test performance should be available before a test is offered in patient care.

Assessing Clinical Utility of Molecular Tests

Clinical utility refers to the usefulness of the test and the value of information to medical practice. In molecular testing, clinical utility represents a balance between health-related benefits and the harms that can occur from a test.[3] In general, the benefits and harms of a molecular test should be compared to the best alternative test to assess incremental benefits and harms. Alternatively, the incremental benefits and harms of using a molecular test should be compared to using no test at all, if that is the current standard of care. Benefits and harms should be considered at multiple levels including the patient, family, healthcare organizations, and society. Each level will have a different perspective of risk, which will ultimately impact the acceptance of a test into routine clinical practice.

Grosse and Khoury apply the framework previously proposed by Fryback and Thornbury[61] to molecular testing.[62] According to these authors, beyond assessing the analytical and clinical validity of a test, the following four levels of impact should be considered: diagnostic thinking,

therapeutic choice, patient outcome, and societal impacts. Diagnostic thinking refers to the value of information in understanding the diagnosis, cause, and prognosis of a condition. Therapeutic choice refers to the use of test results in clinical management of an individual with a diagnosed disorder. Patient outcomes refer to endpoints such as mortality and quality of life, i.e., clinical results that can be perceived by and that matter to the patient.

The Analytic Validity, Clinical Validity, Clinical Utility, and Ethical, Legal, and Social Implications (ACCE) model suggests to consider the following elements when assessing the utility of molecular genetic tests: 1) the natural history of the disorder, 2) availability and effectiveness of interventions, 3) potential adverse outcomes of having the test done, and 4) available resources (education and expertise) to manage all aspects of service.[63]

The impact of interventions that occur as a consequence of a molecular test is particularly important in assessing clinical utility. The impact of an intervention on patient outcomes is ideally measured using randomized controlled trials (RCTs). Although they may have a better internal validity, efficacy RCTs typically focus on short-term outcomes in highly selected patient populations, often making it difficult to generalize the findings. Clinical utility is primarily concerned with effectiveness, which involves measuring long-term health outcomes of the general population in real-world settings. The National Cancer Institute (NCI) is currently sponsoring a pragmatic clinical trial - the TAILORx trial described above. Practical clinical trials involve large sample sizes, broad inclusion criteria, and modest data collection, and provide estimates of effectiveness in typical care settings.[3]

Challenges in Assessing Clinical Utility of Molecular Tests

The major challenge in assessing clinical utility is the lack of studies that directly correlate test results with clinical outcomes. RCTs, particularly effectiveness RCTs, are rarely available. Other study designs, such as case series (single group designs) are prone to various internal validity issues. As a result, evaluation of clinical utility often involves inference based on the evidence for the analytic validity and clinical validity of the test. However, evaluation of analytical and clinical validity itself is also challenging (see our previous discussion). For most LDTs, data on analytical and clinical performance are not publicly accessible unless published in peer-reviewed journals. In contrast, FDA-approved or cleared commercially distributed test kits are accompanied by a kit insert that summarizes the analytical and clinical validity data submitted for approval; FDA Decision Summaries are publicly available via the FDA Web site.

Systematic Reviews

Overall, our searches identified 24 systematic reviews that evaluated the clinical validity or utility of molecular tests. Of the 24 reviews, seven focused on tests for diagnosis of infectious diseases, 12 on tests for diagnosis or treatment monitoring for various cancers, and five on tests used to detect specific gene variants for predicting drug reactions. The purpose, quality, outcomes, and reported findings of the reviews are summarized in Table 30 through Table 32.

Tests for Infectious Diseases

Our searches identified seven systematic reviews that evaluated the clinical validity of molecular tests used for diagnosis of infectious diseases. Important information about these reviews is presented (Table 30). In six of the seven reviews, tuberculosis (TB) was the condition of interest. The remaining review considered the limitations and applications of molecular methods used to diagnose Lyme disease. The six reviews on TB were of high quality, as judged

by the AMSTAR assessment tool.[56,57,64-67] The review on molecular methods for Lyme disease, however, received a low quality rating.[68] Key elements, such as a comprehensive literature search, listing of all included studies, and assessment of included study quality, were either missing or not reported in this review. See Table 29 in Appendix C for further information about the quality of each of the reviews.

All six of the reviews on TB focused on evaluating the evidence on the diagnostic accuracy of nucleic acid amplification (NAA) tests in the diagnosis of tuberculosis. In all of the reviews, PCR was the primary testing method considered. Four of the six reviews considered both laboratory-developed and commercially available tests. However, one review considered only laboratory-developed tests,[66] and one considered only commercially available tests.[64] Evidence on the following commercially available tests was assessed: Amplicor MTB tests (Roche Molecular Systems), the Amplified Mycobacterium Tuberculosis Direct Test (MTD) (Gen-Probe, Inc), BDProbeTecET assay (BD Diagnostic Systems), and LCx (Abbott Laboratories).[3]

The largest and most recent review, published by the U.K.-based National Institute for Health Research (NIHR) in 2007, included 207 studies that evaluated the diagnostic accuracy of PCR methods in patients with suspected TB.[69] Of the 207 studies, 106 used commercial tests and 101 used laboratory-developed tests. The majority of studies included in this review and the other reviews on pulmonary TB used sputum samples for detecting the organism. Similarly, mycobacterial culture was the primary reference standard used in most studies. Some studies, however, used microscopy alone, and some used both culture and microscopy. Below, we summarize the findings of the NIHR review, which appear to be consistent with previous reviews on the diagnostic test accuracy of NAA tests for TB:

- Most of the included studies reported very high estimates of specificity, for both pulmonary and extra-pulmonary TB; sensitivity estimates, in contrast, have been much lower and highly variable.
- Sensitivity estimates have been lower in paucibacillary TB (smear negative and extra-pulmonary TB), and higher in smear positive pulmonary TB.
- There is a lack of consistency in accuracy estimates across studies for both commercial and laboratory-developed tests.
- The sensitivity and specificity of laboratory-developed tests, however, have been more variable and inconsistent than commercial tests. Sensitivity estimates for laboratory-developed tests ranged from 9.4% to 100%, and specificity estimates ranged from 5.6% to 100%. In comparison, for commercially available tests, sensitivity ranged from 62% to 100% and specificity ranged from 98% to 100%.
- The main explanatory factors for the variability were the reference standard used, whether the study was laboratory- or hospital-based, and the use of blinded test interpretation.
- Accuracy appeared to be higher when culture alone was used as a reference standard, the study was laboratory-based, and in studies with lack of blinded interpretation of both index and reference tests. (In another review of laboratory-developed NAA tests,

[3] The LCx (Abbott Laboratories) test has been recently discontinued.

Flores et al. (2005) explored potential sources of heterogeneity, and found that the use of IS6110 target sequence and nested PCR methods appear to significantly increase diagnostic accuracy.[66])

Overall, the authors of the NIHR review and other reviews on NAA tests for TB concluded that diagnostic accuracy must be further established before NAA tests can replace conventional diagnostic tests (e.g., smear and culture) for TB. Future studies should have a prospective design, include a wide spectrum of patients, use appropriate reference tests, and avoid major sources of bias such as verification bias, lack of blinding, and inclusion of indeterminate results.

Note that the Centers for Disease Control and Prevention (CDC) recently updated its guidelines for the use of NAA tests in the diagnosis of TB. Nucleic acid amplification tests can detect Mycobacterium tuberculosis bacteria in specimens one or more weeks earlier than the culture-based conventional tests, and thus could have significant impact on patient care and public health.[70] The new guidelines recommend that NAA testing be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management or TB control activities, such as contact investigations. The recommendation was made based on a report of a panel of clinicians, laboratorians, and TB control officials that was convened by CDC and the Association of Public Health Laboratories.

Tests for Cancers

Our searches identified 12 systematic reviews that evaluated the use of molecular tests for cancer diagnosis or treatment monitoring.[38,43,71-78] Seven of the 12 reviews assessed the clinical utility of molecular tests, with the primary outcomes being recurrence of disease and/or survival. The remaining five reviews evaluated the diagnostic accuracy of molecular test methods to detect the presence of cancer-related genes. In most of the reviews, the studies used PCR as the amplification method. However, the studies included in one review on the use of *ERBB2* gene (also known as *HER2*) testing to manage cancer patients used various commercially available tests[4] that used either fluorescence in situ hybridization (FISH, e.g., PathVysion *HER2* DNA Probe Kit, INFORM *HER2/neu*) or immunohistochemistry (IHC, e.g., PATHWAY).[79] The overall findings and other important information about the reviews are reported in Table 31 in Appendix C. The quality of the reviews ranged from moderate to high. Below, we provide a more thorough discussion of the five most recent reviews. Each review focuses on a different testing method(s): one on various tests using FISH or IHC methods,[79] two on DNA microarrays (including the Onco*type* DX® assay),[38,43] and one on PCR methods (e.g., standard PCR, nested PCR, and real-time PCR).[71]

The first review published by Seidenfield et al. in 2008 evaluated the evidence on the application of *ERBB2* testing for the management of cancer patients.[79] The applications considered included the following: 1) the potential for response to trastuzumab among patients with breast cancer who have negative, equivocal, or discordant *ERBB2* assay results; 2) the use of *ERBB2* assay results to guide selection of breast cancer treatments other than trastuzumab (i.e., chemotherapy); 3) the use of serum *ERBB2* to monitor treatment response or disease progression in patients with breast cancer; and 4) the use of *ERBB2* testing to manage patients with ovarian, lung, prostate, or extracranial cancers. Overall, the review included 71 studies—

[4] See Table 31 for a complete listing of the tests used in the studies included in this review.

four addressed the first application described above, 26 the second application, 15 the third application, and 26 addressed the fourth application. The studies included in the review used FISH or IHC methods to determine *ERBB2* status. While no formal analysis was conducted to assess the concordance and discrepancy of *ERBB2* measurements, the authors do provide a narrative discussion of FISH versus IHC. According to the authors, "there is no recognized gold standard to determine the *HER2* status of tumor tissue, which precludes consensus on one best *HER2* assay."[79]

Overall, the authors of this review indicated that the currently available evidence for the key questions addressed in this review was weak. The evidence was weak on outcomes of trastuzumab added to chemotherapy for most *ERBB2* -equivocal, discordant, or negative patients and for comparing chemotherapy outcomes in *ERBB2* positive and *ERBB2* negative patient subgroups. The evidence was also weak regarding differences by *ERBB2* status for outcomes of chemotherapy for advanced or metastic disease and for testing malignancies of lung, ovary, head and neck, or prostate. The authors concluded that future cancer therapy trial protocols should report the following elements of the potential of *ERBB2* testing to improve treatment outcomes: detailed reporting of how *ERBB2* status was ascertained, stratified randomization by *ERBB2* status or prospectively specified *ERBB2* subgroup analysis of outcomes, and detailed recording of all relevant data and archiving of tissue samples for future subgroup analysis.

A second review published in April 2008 by the Blue Cross Blue Shield Technology Assessment group (TEC) examined the clinical utility of three gene-expression profiling tests—the Onco*type* DX™, MammaPrint® and the Breast Cancer Gene Expression Ratio (also known as the 2-gene ratio or *HOXB13/IL-17BR* ratio).[80] These tests have permitted the analysis of patterns of gene expression in as many as thousands of genes simultaneously. This technology is primarily of interest for its potential use to predict the risk of disease recurrence and to guide the use of adjuvant systemic therapy. Specifically, the TEC report examined whether, compared to conventional risk assessment tools, the use of these tests "improves outcomes when used to decide whether risk of recurrence is low enough to forgo adjuvant chemotherapy for early stage breast cancer."[80]

Overall, the authors concluded that there was insufficient evidence to determine whether MammaPrint® or the Breast Cancer Gene Expression Ratio are better than conventional risk assessment tools in predicting recurrence. However, their analysis of four studies evaluating the Onco*type*DX™ indicated that this test provides "information about the risk of recurrence that is incremental to conventional classifiers used to predict risk. Women classified as high risk by conventional methods and reclassified as low risk by Onco*type*DX™ have a recurrence of at most 10% to 14%." The authors of the TEC report, however, suggest that there are several limitations to the available evidence. In particular, which patient groups benefit the most from the test (low versus moderate versus high risk women) and how the presentation of risk information affects choices is unknown.

In January 2008, the Johns Hopkins Evidence-based Practice Center, under contract to AHRQ, also assessed the analytic validity and clinical validity, and impact on clinical decision making of three gene-expression- based tests – Onco*type* DX™, MammaPrint® and the Breast Cancer Profiling Test (also known as the H/I ratio test).[38] The authors determined that the Onco*type* DX® assay had the strongest evidence for its ability to improve assessment of prognosis than standard risk stratification, at least in ER positive, lymph node negative, tamoxifen-treated women considering adjuvant chemotherapy. However, they point out that

there is still uncertainty about how best to incorporate the test results into decision making, and how best to use the conventional predictors to which it has been compared. They also note that the utility of the risk estimates derived from the test results to *ERBB2* positive patients has not yet been determined. In evaluating studies of the MammaPrint® assay, they noted that it was still unclear who the appropriate target population would be or how much the results would add to decision making based on standard risk factors. They did report that a clinical trial, Microarray in Node-negative Disease (MINDACT), has recently begun in which MammaPrint® will be compared to another method of risk assessment in early stage breast cancer.[38]

Lyman & Kuderer (2006) also examined the evidence on gene expression profile assays as predictors of recurrence-free survival in early stage breast cancer.[43] The primary outcome of interest in this review was disease-free survival based on gene expression risk category. Patients were stratified according to the gene expression profile into a high- or low-risk group. The evidence base for this review consisted of 17 studies that included 2,908 patients ranging from 20 to 668 patients per study. Of the 2,908 patients included in this review, 1,531 (52.6%) were classified as high risk based on the gene assay, and 595 (20.5%) experienced distant breast cancer recurrence during the late period of observation. The reported recurrence rates were 31.2% among gene expression profile patients at high risk, and 8.5% among low-risk patients.

According to the authors of the review, substantial heterogeneity was observed across studies on most measures of test performance. Analysis of test performance measures indicated that the sensitivity of the gene assays for predicting recurrence was relatively high in some studies, but the specificity for identifying those who remain disease free was quite low. The authors suggest that the following may explain study-by-study variation: use of different gene signatures, different risk score cutoff points, and inclusion of different populations of patients with early stage breast cancer (i.e., with respect to disease stage, receptor status, and treatment). Based on the results of their analysis, the authors concluded that gene expression profiles show promise for predicting survival in patients with breast cancer. However, their use in clinical decision making must be considered in light of the between-study variability in assay prognostic performance, specific patient population being evaluated in the included studies (women with early stage breast cancer), and small number of patients included in the studies (50% of studies had fewer than 100 women).[43]

In 2007, Mocellin et al. published a review that evaluated the evidence on the use of PCR to detect the presence of melanoma cells in sentinel lymph nodes (SLN).[71] The authors of the review were primarily concerned with the overall effect of PCR status on patient survival. The evidence base for this review consisted of 22 original articles that included 4,019 patients with stage I or II cutaneous melanoma. Histopathologic examination of SLN consisted of hematoxylin & eosin (HE) staining combined with IHC in all but one study, in which HE only was used. According to the authors of the review, all analyses were performed considering an SLN molecularly positive for melanoma metastasis if at least one tumor marker was observed amplified at PCR analysis. The majority of studies included in the review chose either S-100 or gp100 as tumor markers, and some studies also adopted additional markers (e.g., tyrosinase and/or melanoma antigen recognized by T-cells [MART1]). Ten of the 22 studies used nested PCR, nine used standard PCR, and three used real-time PCR.

According to the authors, meta-analysis of the pooled data of 2,443 patients (not all studies had sufficient data to include in analysis) showed a significantly increased risk of death in

patients with PCR positivity (hazard ratio (HR)[5]: 5.08; 95% CI's 1.83 to 14.08; p = 0.002). Heterogeneity testing, however, indicated significant study variability of effect estimates. Subgroup analysis showed that the findings of two studies caused most of the heterogeneity, with both studies having in common the use of standard PCR and a relatively short follow-up period (<36 months). Further analysis indicated that trials using nested-PCR and longer follow-up periods had the strongest effect in favor of PCR as a prognostic factor. Based on these findings, the authors concluded that PCR status of SLN appears to have a clinically valuable prognostic power in patients with melanoma. However, considering the presence of heterogeneity, the authors warn against overestimating the favorable results of pooled data.[71]

Tests for Predicting Drug Reactions

Our searches identified five systematic reviews that focused on studies of tests used to detect specific gene variants involved in drug metabolism. The quality of the reviews ranged from moderate to high. Table 32 in Appendix C presents important information about the characteristics of the studies included in the reviews and the reported results and conclusions of the reviews. Four of the five reviews evaluated the evidence on a specific metabolic enzyme within the cytochrome P450 (*CYP450*) enzyme system. The *CYP450* family of enzymes is found in the liver and is responsible for metabolizing and eliminating a large number of pharmacologic agents. Polymorphisms of some of the genes within this system are known to impact enzymatic activity. Tests, such as the recently FDA-approved Roche AmpliChip® CYP450 Test, are now available to test for *CYP450* polymorphisms. The AmpliChip® delivers the results of testing for polymorphisms in the form of "predicted phenotypes"—poor metabolizers (PMs), intermediate metabolizers (IMs), extensive metabolizers (EMs), and ultra-rapid metabolizers (UMs). Drug metabolism is thought to be associated with the potential for toxicity or lack of efficacy. In three of the four reviews on *CYP450*, the majority of the included studies used the Roche AmpliChip® CYP450 test to examine polymorphisms.[37,81,82] The authors of the fourth review did not indicate whether the studies used a specific test.[83] The fifth review evaluated the diagnostic accuracy of a commercial line probe assay-INNO-LiPA Rif. TB (LiPA) developed to rapidly detect rifampicin resistance, a marker of multi-drug resistant tuberculosis (MDR-TB).[65]

The first of the five reviews on *CYP450* evaluated the evidence for *CYP2D6* genotyping, compared to no testing, for guiding treatment for patients at high risk for primary breast cancer or breast cancer recurrence.[81] *CYP2D6* plays a major role in the metabolism of tamoxifen, which is prescribed as a component of adjuvant endocrine therapy to prevent breast cancer recurrence, treat metastatic breast cancer, and prevent development of the disease in high risk populations. Eleven studies made up the evidence base for this review—seven studies considered the association of the genotype with plasma levels of active tamoxifen metabolism and four considered the association of the genotype with clinical outcomes. None of the included studies were randomized trials. Overall, the authors of this review concluded that the available evidence was insufficient to determine whether or not the use of *CYP2D6* genotyping for directing endocrine therapy regimen selection improves health outcomes for women at high risk for or with breast cancer. Thus, the evidence was considered insufficient to support the clinical utility of *CYP2D6* testing.

[5] The following is the hazard ratio formula used in the review: observed survived-expected to survive/observed PCR positive-expected PCR positive.

The second review focused on whether the use of *CYP2C19* genotyping to direct treatment of *Helicobacter pylori* (*H. pylori*) infection improved health outcomes compared to no testing (or standard treatment).[82] Polymorphisms in *CYP2C19* affect the metabolism of proton pump inhibitors, which could potentially have an impact on the efficacy of *H. pylori* eradication. Only one randomized controlled trial met the inclusion criteria for this review. The results of the study indicated that *H. pylori* eradication rates at 1 month were 96% in the pharmacogenomics-based treatment group versus 70% in the standard care group (a statistically significant difference, $p < 0.001$). Eradication rates were greater for extensive metabolizers than poor metabolizers. However, because only one study met the inclusion criteria for this review, the authors concluded that the evidence did not permit conclusions on whether the use of a pharmacogenomics-based treatment regimen for *H. pylori* improves eradication rates or net health outcomes.

The third review focused on testing for *CYP450* polymorphisms in adults beginning selective serotonin reuptake inhibitor (SSRI) treatment for non-psychotic depression. The following *CYP450* enzymes are primarily involved in the metabolism of all SSRI's: *CYP2D6*, *CYP2C19*, and *CYP2C9*.[37] The primary question addressed in this review is whether *CYP450* testing would lead to improved clinical outcomes and whether test results were useful in medical, personal, or public health decision-making. Other questions in the review addressed the analytic validity of the *CYP450* test (results discussed in previous chapter), clinical validity of the test (specifically if *CYP450* genotypes predict drug metabolism and efficacy of SSRIs), and adverse events or harms that result from the testing. No studies were identified that directly addressed any aspect of the primary questions of clinical utility. Fourteen studies addressed analytic validity, 16 addressed *CYP450* genotypes and metabolism of SSRIs, five addressed *CYP450* testing and efficacy of SSRIs, and nine studies addressed *CYP450* testing and adverse drug reactions. Pooled analysis of study results was not conducted due to the poor quality of the evidence base and between-study heterogeneity (e.g., healthy volunteers vs. patients and use of SSRIs in patients with conditions other than just depression).[37]

Based on the reported study results, the authors of this review concluded that the data fail to support a clear correlation between CYP polymorphisms and SSRI levels, SSRI efficacy, or tolerability. Further, there are insufficient data regarding whether testing leads to improved outcomes versus no testing in the treatment of depression; whether testing influences medical, personal, or public decision making; or whether any harms are associated with testing itself or with subsequent management.

In the fourth review on the *CYP450* enzyme system, Sanderson et al. (2005)[83] evaluated *CYP2C9* gene variants in patients either starting or already established on warfarin. *CYP2C9* isoenzyme is responsible for the metabolism of a number of drugs, including warfarin, phenytoin, and losartan. The gene coding for *CYP2C9* has been mapped to the long arm of chromosome 10 (10q24.2), within the cluster of *CYP450* genes. Two variants of the *CYP2C9* gene are known to reduce metabolism of warfarin: *CYP2C9*2* by 30% to 50% and *CYP2C9*3* by around 90%. The most serious complication associated with the use of warfarin is bleeding, occurring at a rate of 8 per 100 patients. Individuals with the two identified *CYP2C9* gene variants are thought to be more susceptible to bleeding because of reduced metabolism, and thus may require lower maintenance doses of warfarin.

The evidence base for this review included 11 studies with a total of 3,029 patients. No significant between-study heterogeneity was detected. In all but one study, which did not

report what test method was used, PCR was the testing method. The outcomes considered in this review were drug dose, indicators of anti-coagulation, and bleeding events. Overall, the results of the review showed that patients with *CYP2C9*2* and *CYP2C9*3* alleles have lower mean daily warfarin doses and a greater risk of bleeding. Based on these results, the authors of the review concluded that testing for gene variants could potentially alter clinical management in patients starting treatment with warfarin, but more evidence on the clinical utility and cost-effectiveness of genotyping is needed before routine testing can be recommended.[83]

In the final review, Morgan et al. (2005) assessed the diagnostic accuracy of the commercially available INNO-LiPA Rif. TB kit (LiPA).[65] The LiPA is a line probe assay able to identify the M. tuberculosis complex and simultaneously detect genetic mutations in the *rpoB* gene region related to rifampicin (RIF)-resistance. The test is performed by extracting DNA from cultures or directly from clinical samples and amplifying the RIF-resistance-determining region of the *rpoB* gene using PCR. The M. tuberculosis isolate is considered RIF susceptible if all of the wild-type S probes give a positive signal and all of the R probes react negatively. RIF-resistance is indicated by absence of one or more wild-type S probes. When RIF-resistance is due to one of the four most frequently observed mutations, a positive reaction is obtained with one of the four R probes.

The evidence base for this review included 15 studies with 1,738 specimens. All the studies included in the review reported on the diagnostic accuracy of LiPA. Significant between-study heterogeneity was not detected in this review. Overall, the results showed that the sensitivity was greater than 95% in 12 of 14 studies that applied LiPA to isolates, and the specificity was 100%. In four studies that applied LiPA directly to clinical specimens, the specificity was 100% and the sensitivity ranged from 80% to 100%. Based on these results, the authors concluded that LiPA is a highly sensitive and specific test for the detection of rifampicin resistance in culture isolates. The test, however, appears to have a relatively lower sensitivity when used directly on clinical specimens.

Chapter 4. How Does CLIA Regulate Molecular Testing?

In this chapter, we address how CLIA regulates molecular testing. For this Key Question, we were asked to look at the following aspects of the CLIA regulation:

- Survey components relevant to molecular testing,
- Quality control requirements,
- Proficiency testing,
- Visibility of test claims (labeling),
- Transparency of data used to support test performance, and
- Handling of complaints or unexpected events.

Method

For this question, we reviewed the CLIA regulations and associated guidance documents posted on the Web sites of the CLIA program, FDA, and the Centers for Disease Control and Prevention (CDC). We summarized the information from the current CLIA regulations[84] and the guidance documents published on the Web site of the CLIA program.[85] We also interviewed Penny (Mattingly) Meyers, MA, MT(ASCP)SBB, a staff member in the Centers for Medicare and Medicaid Services (CMS) Division of Laboratory Services of the CLIA program, to discuss CLIA's oversight of molecular tests and verify the accuracy of the summary that we had generated from the CLIA regulations and the guidance documents.

Results

CLIA in Brief

In response to public concerns about the quality of laboratory testing, Congress passed the Clinical Laboratories Improvement Amendments (CLIA) Act in 1988. The goal of this Act was to establish overall quality standards to ensure accuracy, reliability, and timeliness of patient test results regardless of where the test was performed. The final CLIA regulations were published in 1992 and received a major update in 2003. CMS currently assumes primary responsibility for the management of the CLIA program.

Under the CLIA regulations, all facilities that perform tests on "materials derived from the human body for the purpose of providing information for the diagnosis, prevention, or treatment of any disease or impairment of, or the assessment of the health of, human beings" are required to meet certain Federal requirements and must apply and obtain a certificate from the CLIA program. The requirements for CLIA certification are based on the complexity of the tests. FDA has assumed primary responsibility for assigning each test to one of the three CLIA complexity categories: waived tests, tests of moderate complexity and tests of high complexity. Under the CLIA regulation, tests may be waived from oversight if they meet certain requirements. Waived tests are defined as simple laboratory examinations and procedures that are cleared by the Food and Drug Administration (FDA) for home use; or that employ methodologies that are so simple and accurate as to render the likelihood of erroneous results negligible; or that pose no

reasonable risk of harm to the patient if the test is performed incorrectly. Detailed criteria for categorizing a test as waived are listed in the CLIA regulations at 42 CFR 493.15(b) and 493.15(c), as well as on FDA's Web site (http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/IVDRegulatoryAssistance/ucm124208.htm, accessed on December 3, 2009).

There are currently five types of CLIA certificates: Certificate of Waiver (COW), Certificate for Provider Performed Microscopy (PPM) Procedures, Certificate of Registration (COR), Certificate of Compliance (COC) and Certificate of Accreditation (COA). COW is issued to a laboratory that performs only waived tests. A certificate for PPM Procedures is issued to a laboratory in which a physician, midlevel practitioner or dentist performs specific microscopy procedures during the course of a patient's visit. A limited list of microscopy procedures is included under this certificate type and these are categorized as moderate complexity. Laboratories that have a COW or PPM certificate are not subject to routine CLIA surveys (inspections).

COC is issued to a laboratory that performs nonwaived (moderate and/or high complexity) testing once the State surveyor conducts a survey (inspection) and determines that the laboratory is compliant with all applicable CLIA requirements. A laboratory that performs nonwaived (moderate and/or high complexity) testing can also apply for a COA in lieu of COC if the laboratory is accredited by one of the six accreditation organizations approved by CMS (described later in this chapter). To obtain a COC or COA, a laboratory is required to undergo biennial surveys and must meet the CLIA quality standards for quality control (QC), proficiency testing (PT), quality assurance (QA), personnel qualifications, and specimen integrity/recordkeeping. (The requirements will be discussed in detail in the following sections).

Certificate of Registration is issued to a laboratory that has applied for a COC or COA, but the survey has not been completed by the State Department of Health or the accrediting organization. The issuance of Certificate of Registration allows the laboratory to conduct nonwaived (moderate and/or high complexity) testing until the laboratory is surveyed and determined to be compliant with the CLIA regulations.

Under CLIA, clinical tests are categorized under different laboratory specialties (e.g., microbiology, hematology and pathology) and subspecialties (e.g., bacteriology, virology, histopathology and cytology). The specialties and subspecialties under which a laboratory can perform tests are specified on the COC or COA. A laboratory may need to meet additional requirements to be certified to perform tests under these specialties and subspecialties.

In addition, section 353(p) of the Public Health Service Act provides for the exemption of laboratories from the requirements of CLIA when the State in which they are located has requirements equal to or more stringent than those of CLIA. Currently, two States—Washington and New York—have CLIA-exempt status.[86]

Molecular Testing Regulation under CLIA

Like any other clinical tests, molecular tests are subject to all general CLIA rules. Since molecular tests are normally categorized as either moderate or high complexity tests, a laboratory performing molecular tests is required to apply for and obtain a COC or a COA. Currently, there is no CLIA specialty or subspecialty for molecular or biochemical genetic testing. Therefore, there are no special personnel, quality control, or proficiency-testing requirements for molecular

tests unless the laboratory voluntarily chooses a CMS-approved accrediting organization specifying additional requirements. In the following sections, the general CLIA requirements applicable to molecular tests are described.

Survey Components Relevant to Molecular Testing

To obtain a COC or a COA to perform molecular tests, a laboratory is required to be surveyed (inspected) by a CLIA-authorized State agency (for COC) or an accrediting organization (for COA). All general survey components described below are applicable to molecular tests. We did not identify any molecular test-specific survey components. Unless specified otherwise, the content presented in this section is based on the document, *Policy for Conducting Surveys*, published on the CMS's CLIA program Web site.[87]

Under CLIA, a laboratory may be surveyed for different reasons. To perform moderate/high complexity tests like molecular tests, a laboratory must first undergo an initial survey for certification (COC or COA), and then be surveyed biennially for recertification. The laboratory may also be surveyed for complaint (on an unannounced basis), follow-up, and/or validation (for those holding a COA).

The focus of CLIA surveys is to assess how a laboratory monitors its operations and ensures the quality of its testing. Each certification/recertification survey includes a tour of the facility, record review, observation, and interviews with personnel involved in the pre-analytic, analytic, and post-analytic phases of the testing process. The same survey guidelines and protocols published by CMS[87] are used by the State agency surveyors to increase consistency in the survey process, although the professional judgments of the surveyors are also a critical element in the process.

Before or during a survey, the surveyors collect and review a large amount of information regarding the laboratory's operation, personnel, quality control, quality assurance system, and proficiency testing (Table 2). During the survey, the surveyors will observe the laboratory's physical layout, specimen collection and processing, and the working areas for preparation, testing, reporting, and storage. The focus of the observation is on specimen integrity, quality control performance, skills and knowledge of personnel regarding testing, adequacy of staffing for test volume, and interactions and communication among personnel. The surveyors also observe and verify that reagents, kits, and equipment correlate with the test menu, clients served, and results reported.

During the survey, surveyors may interview staff to confirm observations and obtain additional information, as necessary. Surveyors may also use the interviews as an opportunity to evaluate the laboratory personnel's knowledge and skills for performing tests and identifying problems and the methods for corrective and remedial actions.

Table 2. Information Collected and Reviewed for CLIA Certification/Recertification Surveys

- Services offered—the list of tests and specialties/subspecialties
- Standard operating procedure manual with all test procedures (e.g., package inserts and supplemental information, as necessary)
- Reference laboratory's client services manual, if applicable
- Records of tests referred to other laboratories
- Personnel records, including diplomas, certificates, degrees, training and experience, continuing education, competency assessment, duties/responsibilities, and personnel changes
- Quality control records, including remedial action information, calibration and calibration verification records, statistical limits, and instrument maintenance and function checks records
- Proficiency testing (PT) reports, including test runs with PT results, direct printouts, and remedial actions for unsatisfactory results
- Quality system assessment plan and documentation; for each of the systems:
    - Policies and procedures to monitor, assess, and correct identified problems
    - Documentation of ongoing assessment activities, including review of the effectiveness of corrective actions taken, revision of policies and procedures to prevent recurrence of problems, and discussion of assessment reviews with staff
- Safety information
- Patient testing records including requisition (patient charts may be used), work records (direct printouts), and patient test reports (patient charts may be used)

Quality Control Requirements

Quality control (QC) consists of the procedures used to detect errors that occur due to test system failure, adverse environmental conditions and variance in operator performance, as well as the monitoring of the accuracy and precision of the test performance over time.[88] Subpart K of the CLIA regulations defines the general quality system for nonwaived testing. Section 493.1256 particularly describes in detail the requirements for quality control procedures. Table 3 is a summary of the key QC requirements that a laboratory performing nonwaived tests (including molecular tests) must meet.

Table 3. Key Quality Control Requirements Relevant to Molecular Testing

- For each test system, the laboratory is responsible for having control procedures that monitor the accuracy and precision of the complete analytical process.(42 CFR 493.1256 (a)) The control procedures must:
  - detect immediate errors that occur due to test system failure, adverse environmental conditions, and operator performance (42 CFR 493.1256 (c)(1)) and
  - monitor over time the accuracy and precision of test performance that may be influenced by changes in test system performance and environmental conditions, and variance in operator performance. (42 CFR 493.1256 (c)(2))
- The laboratory must establish the number, type, and frequency of testing control materials using, if applicable, the performance specifications verified or established by the laboratory. (42 CFR 493.1256 (b))
- Each laboratory that introduces a test system not subject to FDA clearance or approval—including methods developed in-house—must, before reporting patient test results, establish for the test system the performance specifications for the following performance characteristics, as applicable:
  - accuracy,
  - precision,
  - analytical sensitivity,
  - analytical specificity to include interfering substances, reportable range of test results for the test system,
  - reference intervals (normal values), and
  - any other performance characteristic required for test performance. (42 CFR 493.1253 (b)(2))
- For test systems developed in-house, a laboratory must establish a maintenance protocol, and perform and document the maintenance activities, to ensure test system performance to produce accurate and reliable test results (42 CFR 493.1254 (b)).
- Each test system that has an extraction phase, include two control materials, including one that is capable of detecting errors in the extraction process. (42 CFR 493.1256 (d)(3)(iv))
- Each molecular amplification procedure, include two control materials and, if reaction inhibition is a significant source of false negative results, a control material capable of detecting the inhibition. (42 CFR 493.1256 (d)(3)(v))

Proficiency Testing

The information provided in this section is from Subparts H and I of the CLIA regulations, unless specified otherwise.

Under CLIA, each laboratory performing nonwaived testing (including molecular tests) must enroll in one CMS-approved proficiency testing (PT) program for each specialty, subspecialty, and analyte specified in Subpart I of the CLIA regulations. The purpose of PT is to externally evaluate the quality of a laboratory's performance. A sample of (normally five) PT specimens are sent to the laboratories holding a COC three times a year.[3] The PT survey specimens contain analytes known to the suppliers but not to the recipient laboratories. The sample specimens must be tested following the same procedures that the laboratory would use to test patient specimens. If a laboratory fails to perform successfully in a CMS-approved PT program, CMS may direct the laboratory to undertake personnel training or to obtain technical assistance, or it may impose various sanctions, including cancellation of Medicare/Medicaid reimbursement, suspension, limitation or revocation of the CLIA certificate.

Molecular tests are not listed in Subpart I, therefore laboratories are not required to participate in a formal PT program for molecular tests. (However, an accredited laboratory may still be required by the accreditation organization to participate in the available PT programs) Under CLIA, a subspecialty of clinical cytogenetics is established under the cytology specialty but this subspecialty is limited to chromosomal analysis and does not include molecular tests.[89] Although laboratories can choose to enroll in other specialties (e.g., pathology), they are not required to do so. Meanwhile, no PT programs are mandated for the pathology specialty (except for the subspecialty of cytology, which is limited to gynecologic examinations) or for the clinical cytogenetics subspecialty under current regulations.

Nonetheless, although laboratories are not required to participate in a PT program for molecular tests, they are required by CLIA to establish and maintain the accuracy for the molecular tests that they perform. According to Section 93.1236 (c) of the CLIA regulations, at least twice annually laboratories must verify the accuracy of any test or procedure it performs for which participation in a CMS-approved PT program is not mandatory. To verify the accuracy of molecular tests, laboratories may use methods of their own choosing, which may include participation in a voluntary (i.e., not-CMS-approved) PT program and exchanging samples with other laboratories for cross-validation of test results. Laboratories are also required to document the activities of molecular test accuracy verification and make the data available for onsite survey.

Visibility of Test Claims (Labeling)

We did not identify any requirements in the CLIA regulations (including relevant guidance published by CMS) for laboratories to submit data to support claims of performance (analytic or clinical validity or clinical utility). However, there are requirements relevant to analytical and clinical performance. As described previously in Table 3, before reporting patient test results, each laboratory must have established for each laboratory-developed test system introduced after April 24, 2003, the performance specifications for accuracy, precision, analytical sensitivity, analytical specificity to include interfering substances, reportable range of test results for the test system, reference intervals (normal values), and any other applicable performance characteristic required for test performance (42 CFR 493.1253).

Transparency of Data Used to Support Test Performance

We identified requirements regarding transparency of data to support test performance in various sections of the CLIA regulations. According to the regulations, a laboratory must:

- Document all activities related to establishment and verification of performance specifications in accuracy, precision, analytical sensitivity, analytical specificity to include interfering substances, reportable range of test results for the test system, reference intervals (normal values), and any other performance characteristic required for test performance (42 CFR 493.1253(c)).
- Document all preanalytic (42 CFR 493.1249 (c)), analytic (42 CFR 493.1289(c)) and postanalytic (42 CFR 493.1299(c)) system assessment activities.
- Provide, upon request, all information and data needed by CMS or a CMS agent to make a determination of the laboratory's compliance with the applicable requirements of the CLIA regulations (42 CFR 493.1773 (d)).

CMS may disclose accreditation organization inspection results to the public if the results are related to an enforcement action (42 CFR 493.571 (a)). CMS may also disclose the results of all inspections conducted by CMS or its agent (42 CFR 493.571(c)). Disclosure of State inspection results is the responsibility of the approved State licensure program, in accordance with State law (42 CFR 493.571 (b)).

Handling of Complaints

The findings presented in this section regarding how complaints are handled under CLIA are based on Subparts Q and R of the CLIA regulation.

A complaint against a laboratory is an allegation that could result in citing noncompliance with any of the CLIA requirements. Upon receiving a complaint, the CMS's State Agency (SA) or regional office will start an investigation, regardless of the type of CLIA certificate the laboratory has or whether it is State-exempt. The investigation can be conducted by an unannounced onsite survey, by telephone, by electronic communication, by letter, or by a documentary review. If the complaint is substantiated, i.e., sufficient evidence is found to conclude that noncompliance exists at the time of the investigation, CMS will take subsequent actions against the laboratory depending on the severity and nature of the deficiencies cited and the facility's willingness or ability to correct them. The various sanctions that CMS may impose on the laboratory include enforcement of a plan of correction (POC), State onsite monitoring, civil money penalty, loss of Medicare reimbursement, suspension, limitation or revocation of the CLIA certificate, and civil and criminal suits.

If the cited deficiencies pose immediate jeopardy, CMS requires the laboratory to take immediate action to remove the jeopardy and may impose one or more sanctions to help bring the laboratory into compliance. If the findings of a revisit indicate that the laboratory has not eliminated the jeopardy, CMS suspends, limits, or even revokes the laboratory's CLIA certificate In addition, if CMS has reason to believe that the continuation of any activity by any laboratory (either the entire laboratory operation or any specialty or subspecialty of testing) would constitute a significant hazard to the public health, CMS may bring suit and seek a temporary injunction or restraining order against continuation of that activity by the laboratory.

If the laboratory has condition-level deficiencies—i.e., serious deficiencies in CLIA's terminology—that do not pose immediate jeopardy, CMS may cancel the laboratory's approval to receive Medicare payment for its services, suspend, limit, or revoke the laboratory's CLIA certificate, or impose any other sanctions previously mentioned until a revisit indicates that the deficiencies have been corrected. However, if the laboratory has deficiencies that are not at the condition level—i.e., less serious deficiencies, it must submit a plan of correction that is acceptable to CMS in content and time frame. If it is found on a revisit that the laboratory has not corrected the deficiencies within 12 months after the last day of inspection, CMS cancels the laboratory's approval to receive Medicare payment for its services and notifies the laboratory of its intent to suspend, limit, or revoke the laboratory's CLIA certificate and of the laboratory's right to a hearing.

In addition, according to 42 CFR 493.1233, each laboratory must have a system in place to ensure that it documents all complaints and problems reported to the laboratory. The laboratory must conduct investigations of complaints, when appropriate.

Clinical Validity or Utility of Laboratory Testing

The terms "clinical validity" and "clinical utility" are not explicitly mentioned in the CLIA regulations. However, we identified the following requirements in the regulations that might be interpreted by some stakeholders as the mechanisms to ensure clinical validity or utility of tests:

- The laboratory director must ensure that testing systems developed and used for each of the tests performed in the laboratory provide quality laboratory services for all aspects of test performance, which includes the preanalytic, analytic, and postanalytic phases of testing (42 CFR 493.1445(e)(1))
- The laboratory director must ensure that the test methodologies selected have the capability of providing the quality of results required for patient care. (42 CFR 493.1445(e)(3)(i)) The clinical consultant provides consultation regarding the appropriateness of the testing ordered and interpretation of test results. The clinical consultant must:
    (a) Be available to provide clinical consultation to the laboratory's clients;
    (b) Be available to assist the laboratory's clients in ensuring that appropriate tests are ordered to meet the clinical expectations;
    (c) Ensure that reports of test results include pertinent information required for specific patient interpretation; and
    (d) Ensure that consultation is available and communicated to the laboratory's clients on matters related to the quality of the test results reported and their interpretation concerning specific patient conditions. (42 CFR 493.1419)

These requirements do not specify what types of data are appropriate for establishing clinical relevance of the tests, where the data should come from (e.g., from research carried out by the laboratory itself or from data reported in peer-reviewed literature), and how the data should be synthesized to reach conclusions.

In June 2009, the Clinical Laboratory Improvement Advisory Committee (CLIAC) Genetic Testing Good Laboratory Practices Workgroup published a report, providing a series of

recommendations for ensuring quality of genetic testing for heritable diseases and conditions.[4] The recommendations were made to CLIAC, an advisory entity that provides recommendations to the Department of Health and Human Services on approaches to ensuring the quality of genetic testing. Although genetic testing for heritable diseases or conditions is beyond the scope of this Horizon Scan, most of the recommendations that the Workgroup made on laboratory practice are relevant to the purpose of the report. The following are the Workgroup's recommendations relevant to clinical validity or utility of molecular testing:

- Laboratories should ensure that the molecular genetic tests they perform are clinically usable and can be interpreted for specific patient situations. Laboratory responsibilities for clinical validity include the following:
    - Documenting information regarding clinical validity (including clinical sensitivity, clinical specificity, positive predictive value, and negative predictive value) of all genetic tests the laboratory performs from available information sources (e.g., published studies and professional practice guidelines)
    - Providing clinical validity information to users of laboratory services before tests are selected and specimens submitted
    - If clinical validity information is not available from published sources, establishing clinical sensitivity, clinical specificity, and predictive values on the basis of internal study results
    - Documenting whether the clinical claims in the references or information sources used can be reproduced in the laboratory and providing this information to users, including indicating test limitations in all test reports
    - Informing users of changes in clinical validity values as a result of knowledge advancement
    - Specifying that the responsibilities of the laboratory director and technical supervisor include ensuring appropriate documentation and reporting of clinical validity information for molecular genetic tests performed by the laboratory
- Directors of laboratories that perform molecular genetic testing for heritable diseases and conditions must fulfill the CLIA responsibility requirements. In addition, these laboratory directors should be responsible for the following:
    - Ensuring documentation of the clinical validity of any molecular genetic tests the laboratory performs, following the recommended practices
    - Ensuring the specimen retention policy is consistent with the laboratory quality assessment activities

The Role of Accreditation Organizations and Exempted States under CLIA

According to the CLIA regulations (42 CFR 493.551(a)), CMS may deem a laboratory to meet all applicable CLIA program requirements through accreditation by a private nonprofit accreditation program (that is, grant deemed status), or may exempt from CLIA program requirements all State-licensed or -approved laboratories in a State that has a State licensure program established by law, if the following conditions are met:

(1) The requirements of the accreditation organization or State licensure program are equal to, or more stringent than, the CLIA condition-level requirements specified in this part, and the laboratory would meet the condition-level requirements if it were inspected against these requirements.

(2) The accreditation program or the State licensure program meets the requirements of this subpart and is approved by CMS.

(3) The laboratory authorizes the approved accreditation organization or State licensure program to release to CMS all records and information required and permits inspections as outlined in this part.

According to the CMS CLIA program (http://www.cms.hhs.gov/clia/), currently there are six CLIA-approved accreditation organizations, including AABB (formerly known as the American Association of Blood Banks), American Osteopathic Association (AOA), American Society of Histocompatibility and Immunogenetics (ASHI), COLA (formerly known as the Commission on Office Laboratory Accreditation), College of American Pathologists (CAP), and the Joint Commission. Based on the same source of information, two States—Washington and New York—currently have CLIA-exempt status.

These private organizations and exempted states play an important role under CLIA in ensuring the quality of laboratory testing, particularly molecular testing. For example, CAP requires laboratories in their Laboratory Accreditation Program to demonstrate the analytic validity of these tests as well as to document how they are clinically validated.[90] CAP's Laboratory Accreditation Program has a specialty inspectors list for molecular diagnostics. Inspectors are reviewed for qualifications in the four main areas of molecular testing—infectious disease, hematology/hematopathology, solid tumors, and heritable diseases—and selected on this basis.[90] CAP's accreditation program has established criteria specifically for molecular testing—including its Molecular Pathology Checklist—to ensure the quality of LDMTs and modified FDA kits.[91] Working with other organizations, CAP has also established PTs for some molecular tests (discussed in Chapter 7).

As a CLIA-exempt State, New York has a unique regulatory process for laboratory testing. Under the State law, a laboratory must be pre-approved by the State if the laboratory intends to perform any testing on a New York resident, regardless of whether the laboratory is located within or outside of the State's territory.[3] Because of the law, many laboratory tests performed by reference laboratories from other States have been reviewed by the Clinical Laboratory Evaluation Program (CLEP) of New York State.[3] CLEP's review process requires laboratories to demonstrate that a test is validated both analytically and clinically prior to being introduced.[92] CLEP also has some specific standards for molecular testing, including requirements for clinical information about test selection and interpretation, patient consent, confidentiality, specimen retention times, quality control procedures, method documentation, and retention of records.[92]

Chapter 5. What FDA Guidance Has Been Issued Pertaining to Oversight of Laboratory-Developed Molecular Testing?

In this chapter we address the following Key Question 5: What FDA guidance has been issued pertaining to oversight of in-house molecular testing?

Methods

To address this question, we searched the FDA's online database containing the guidance documents published by the Agency. We further interviewed Dr. Steve Gutman, former Director of the Office of In-Vitro Diagnostic Device Evaluation and Safety of the FDA, to discuss the oversight of molecular tests by the Agency and verify the accuracy of the guidance documents list that we had generated based on the information from the FDA Web site.

Results

We identified two FDA guidance documents relevant to laboratory-developed molecular tests (LDMTs). These two documents—one guidance for analyte specific reagents (ASRs) and a draft guidance for in-vitro diagnostic multivariate index assays (IVDMIAs)—address the oversight of laboratory-developed tests (LDTs) and are applicable to LDMTs. In addition, we identified several FDA guidance documents regarding commercially distributed genetic testing systems, which are not directly applicable to LDMTs but provide useful information on how the FDA assesses the performance of molecular tests.

FDA Guidance for ASRs

On September 14, 2007, FDA issued a guidance document for industry and FDA staff to clarify the regulations regarding commercially distributed ASRs.[33] The ASR guidance document clarified that ASRs are medical devices that are regulated by FDA. Most ASRs are classified as Class I devices subject to general controls, but exempt from premarket notification requirements.

FDA defines ASRs as "antibodies, both polyclonal and monoclonal, specific receptor proteins, ligands, nucleic acid sequences, and similar reagents which, through specific binding or chemical reactions with substances in a specimen, are intended for use in a diagnostic application for identification and quantification of an individual chemical substance or ligand in biological specimens." In the guidance document, FDA provided some molecular test-related ASR examples. These examples include single forward/reverse oligonucleotide primers and nucleic acid probes intended to bind a single complementary amplified or unamplified nucleic acid sequence, if these reagents are marketed without clinical or analytical performance claims. FDA also provided examples of reagents not considered as ASRs, such as products that include more than a single ASR, control materials or calibrators, microarrays, and products with specific performance claims, interpretation for use, or software for interpretation of results.

The ASR guidance allows only physicians and other persons authorized by applicable State law to order LDTs that are developed using ASRs. The guidance requires the laboratory that

develops an LDT using an ASR to add a statement disclosing that the laboratory developed the test and it has not been cleared or approved by FDA when reporting the test result to the practitioner. The ASR guidance also prohibits advertising and promotional materials for ASRs from making any claims for clinical or analytical performance. Manufacturers who wish to make analytical and/or clinical performance claims for a product must submit an application to FDA for premarket review rather than marketing the product as an ASR.

FDA Draft Guidance for IVDMIAs

On July 26, 2007, FDA published a draft guidance document on IVDMIAs for industry, clinical laboratories, and the FDA staff.[93] In the document, FDA defined an IVDMIA as a device that:

- Combines the values of multiple variables using an interpretation function to yield a single, patient-specific result (e.g., a "classification," "score," "index," etc.), that is intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment or prevention of disease, and
- Provides a result whose derivation is non-transparent and cannot be independently derived or verified by the end user.

By this definition, some LDMTs will fall into the category of IVDMIA. The examples of IVDMIA listed in the FDA guidance document include a gene expression profiling assay for breast cancer diagnosis, a device that integrates quantitative results from multiple immunoassays to obtain a qualitative "score" that predicts a person's risk of developing a disease or condition, and a device that integrates a patient's age, sex, and genotype of multiple genes to predict risk of or diagnose a disease or condition. FDA would not consider devices with a function that simply facilitates the interpretation of multiple variables that health care practitioners could otherwise interpret themselves to be IVDMIAs.

FDA believes that most IVDMIAs—either as commercial kits or in-house testing systems—will be Class II or III device and, therefore, will require a 510(k) clearance or PMA before being marketed. Like other in-vitro devices (IVDs), IVDMIAs are subject to FDA's labeling and postmarket requirements, and can be used as investigational devices or humanitarian use devices if they meet certain conditions. Based on the proposed guidance, for a LDMT that qualifies as an IVDMIA, a developer will need to submit data to back up the claims regarding analytical and clinical performance.

Note that the FDA's IVDMIA Draft Guidance is not a finalized document. As such, this draft guidance only represents FDA's current thinking on this topic. FDA's oversight of these devices has not yet been implemented or articulated in a final guidance document.

FDA Guidance for Commercial Kits or Testing Materials

FDA has so far issued a series of guidance documents for commercially distributed genetic testing kits. These documents do not address the oversight of LDMT and some of them pertain to heritable conditions, which are not within the scope of our report. However, these documents are relevant to the report because they describe how FDA assesses the safety and performance of molecular tests, particularly in the domains of pre-analytical factors, quality control, analytical performance, and clinical validation. Table 4 is a list of these guidance documents for further reference.

Table 4. Published FDA Guidance Documents Related to Molecular Test Kits or Materials

Title	Date of Issuance
Guidance for industry: In the manufacture and clinical evaluation of In Vitro tests to detect nucleic acid sequences of human immunodeficiency viruses types 1 and 2[94]	December 1999
Guidance for Industry and FDA staff: Replacement Reagent and Instrument Family Policy[95]	December 11, 2003
Guidance for Industry and FDA staff: Class II Special Controls Guidance Document: factor V Leiden DNA mutation detection systems[96]	March 16, 2004
Guidance for Industry and FDA staff: Class II Special Controls Guidance Document: Drug Metabolizing Enzyme Genotyping System[97]	March 10, 2005
Guidance for Industry and FDA staff: Class II Special Controls Guidance Document: Instrumentation for Clinical Multiplex Test Systems[98]	March 10, 2005
Guidance for Industry and FDA staff: Class II Special Controls Guidance Document: Automated Fluorescence *in situ* Hybridization (FISH) Enumeration Systems[99]	March 23, 2005
Guidance for Industry and FDA staff: Class II Special Controls Guidance Document: RNA Preanalytical Systems (RNA Collection, Stabilization and Purification Systems for RT-PCR used in Molecular Diagnostic Testing)[100]	August 25, 2005
Guidance for Industry and FDA staff: Class II Special Controls Guidance Document: CFTR Gene Mutation Detection Systems[101]	October 26, 2005
Draft guidance for industry and FDA staff: Nucleic Acid Based In Vitro Diagnostic Devices for Detection of Microbial Pathogens (Draft)[102]	December 8, 2005
Guidance for industry and FDA staff: Class II Special Controls Guidance Document: Quality Control Material for Cystic Fibrosis Nucleic Acid Assays[103]	January 10, 2007
Guidance for industry and FDA staff: Class II Special Controls Guidance Document: Class II Special Controls Guidance Document: Gene Expression Profiling Test System for Breast Cancer Prognosis[104]	May 9, 2007
Guidance for industry and FDA staff: Pharmacogenetic Tests and Genetic Tests for Heritable Markers[105]	June 19, 2007
Guidance for Industry and FDA Staff: Class II Special Controls Guidance Document: Nucleic Acid Amplification Assay for the Detection of Enterovirus RNA[106]	January 2, 2009

Chapter 6. What Is the Role of Other Federal Agencies in Regulating Marketing Claims Regarding the Clinical Validity and Utility of Laboratory-Developed Tests Not Currently Being Actively Regulated by FDA?

This chapter addresses Key Question 6. For this question, we were asked to examine the role of other Federal agencies in regulating marketing claims regarding the clinical validity and utility of laboratory-developed tests not currently being actively regulated by FDA.

Methods

To address this question, we included information from the Federal Register and other sources of Federal guidance documents. We also performed a literature search of MEDLINE and other suitable databases containing primary literature relevant to this question (Appendix A). We further discussed the results of our literature search with Dr. Steve Gutman while he was Director of the Office of In-Vitro Diagnostic Device Evaluation and Safety of the FDA, to confirm that we had identified the relevant sources.

Results

Our literature search and interview with Dr. Gutman identified that, in addition to FDA, the U.S. Federal Trade Commission (FTC) may play a role in regulating marketing claims regarding the clinical validity and utility of LDMTs. No other government agencies were identified.

Regulation of marketing claims regarding the clinical performance of medical devices involves oversight of both labeling and advertising. Device labeling covers a broad category of materials including brochures, mailings, journal reprints if distributed by (or on behalf of) a company, sales materials, package inserts, and immediate package label. Advertising is not defined in the Food, Drug and Cosmetic Act. However, the Center for Drug Evaluation and Research of FDA has a technical definition of advertisement, which includes all ads in published journals and magazines, other periodicals and broadcast ads.[107]

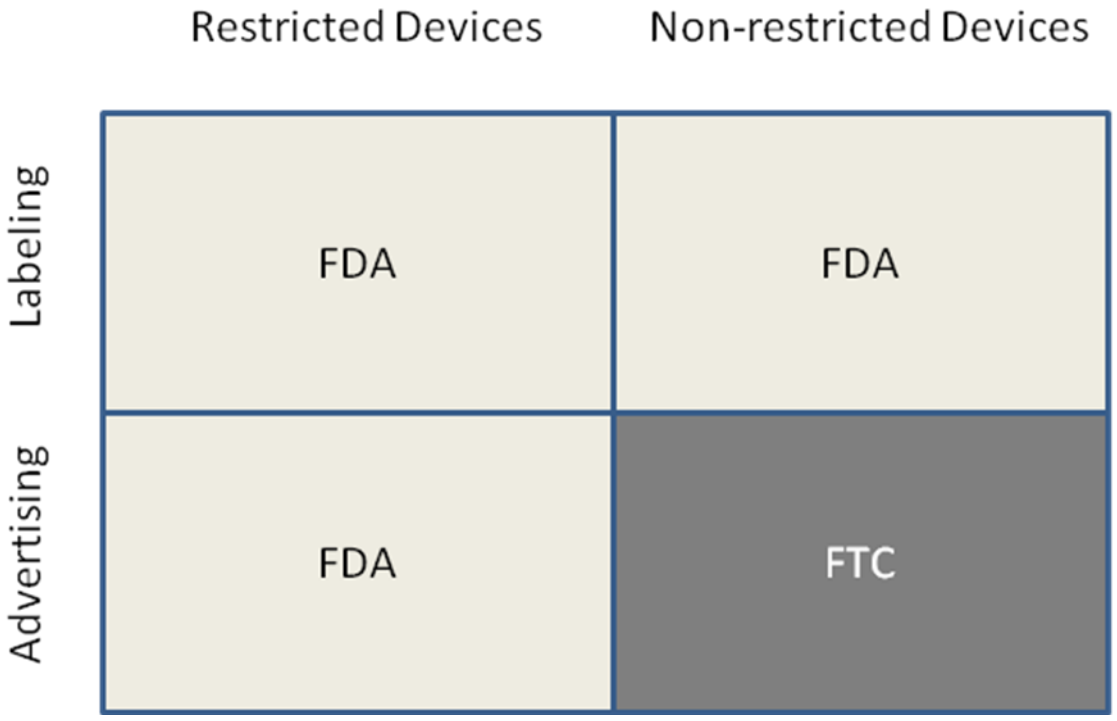
Currently, both FDA and FTC play a role in regulating marketing claims of medical devices. According to a FTC-FDA Memorandum of Understanding in 1971,[108] FDA has primary jurisdiction over advertising of prescription drugs and of restricted devices—i.e., the devices whose sale, distribution or use is restricted by FDA-approval order or by regulation—and over labeling of all products. FTC has primary jurisdiction over advertising of non-restricted devices and of over-the-counter (OTC) drugs. One potential loophole in this two-agency regulation system is the marketing of products on the Internet. FDA and FTC have not clearly defined Internet promotion as labeling or advertising. However, FDA may soon take steps to address this issue. Recently, FDA announced that, in November 2009, the agency would hold a public hearing on the promotion of drugs and medical devices on the Internet and other new media tools.[109]

Under this FTC-FDA regulation framework, both Agencies theoretically have a role in overseeing marketing claims regarding the performance of LDMTs. The oversight of LDMT

labeling is under FDA's jurisdiction. The oversight of advertising regarding LDMTs is also under FDA's jurisdiction if the sale, distribution or use of the LDMTs is restricted by FDA-approval order or by regulation—i.e., these LDMTs are restricted devices. For non-restricted LDMTs, FTC assumes the primary responsibility in the oversight of advertising.

FTC can also take actions against providers of unrestricted LDMTs (i.e., the devices whose sale, distribution or use is not restricted by FDA-approval order or by regulation), when these providers' advertisements make false or misleading claims regarding the clinical performance of LDMTs. Almost all LDMTs can currently be viewed as non-restricted devices. However, the fact that FDA exercises the oversight discretion on LDMTs affects FTC's ability to act against false and misleading advertising regarding such tests, because FTC often uses FDA's labeling requirements as guidance regarding whether a claim is false or misleading.[110] Figure 1 is a depiction of the roles played by FTC and FDA in oversight of medical device marketing claims.

Figure 1. Oversight of Medical Device Marketing Claims



Chapter 7. How Is Proficiency Testing Accomplished for Molecular Tests?

For Key Question 7, we were asked to take a closer look at how proficiency testing is accomplished for molecular tests, specifically in the following areas:

- Control materials
    - What programs exist to provide human mutation samples or synthetic samples for use as control materials?
    - To what extent do laboratories exchange control samples voluntarily for the purpose of proficiency testing?
- What method-specific (rather than analyte-specific) proficiency tests have been developed?
- What organizations or programs are implementing proficiency testing programs for molecular tests, and what standards have these programs established?
- What proficiency test results for molecular testing have been published by the identified testing organizations?
- Based on these published studies, what factors have been identified which contribute to variability within and across laboratories performing molecular testing?
- Based on published studies, what impact have these proficiency testing programs had on reducing variation within and between laboratories?
- What data are available from the accrediting organizations on the impact of proficiency testing programs on laboratory performance?

Methods

To address this question, we summarized information relevant to proficiency testing of laboratories performing molecular tests, such as availability of control samples and method-specific proficiency testing. We summarized published studies of proficiency testing, with attention to factors identified in these studies as contributing to variation between laboratories and evidence for the impact of testing programs on performance.

Systematic reviews relevant to the questions were given highest priority followed by other publication types, including evaluation studies. Recent narrative reviews and an interview with a CLIA staff member (see the method section of Chapter 4) were utilized, as necessary, to address questions not covered by systematic reviews or primary studies. Meeting abstracts, Web-based publications, and other "gray literature" were also searched when published literature was not available to address the questions.

Results

Published Studies

A proficiency testing program is an essential component of external quality assurance. The organizer of the program routinely sends samples to participating laboratories, which test the samples and return results to the organizer. The organizer uses the results to monitor and improve the performance of the participating laboratories.

We identified 33 published studies of proficiency testing, and no systematic reviews of proficiency testing (Table 5). These studies will be discussed throughout this section. For full details of the published studies, refer to Table 33 and Table 34 in Appendix C.

Table 5. Overview of Published Studies of Proficiency Testing

Type of Publication	Test Type(s)	Number of Publications Identified	References
Systematic review	Any	0	
Reports of proficiency test programs	FISH	4	111-114
	PCR, general	6	115-120
	PCR, infectious diseases	16	121-136
	PCR, oncology	3	137-139
	DNA sequencing	4	140-143

Control Materials

The National Institute of Standards and Technology (NIST) and the World Health Organization (WHO) both create, validate, and distribute primary standards for diagnostic assays. Primary standards are held by an independent organization and are reference materials that have been qualified at the highest level. Primary standards for molecular tests are, however, rare. WHO has developed primary standards only for hepatitis B, hepatitis C, and HIV, and the coagulation disorder caused by the Factor V Leiden mutation.[29,30] The only primary standards for molecular tests that NIST has developed are three standards for use in forensic and paternity testing and one standard for fragile X assays (www.nist.gov).[22] However, these tests are beyond the scope of this report.

Laboratories must be able to obtain controls, validation specimens, and proficiency test materials for molecular tests. Controls preferably have been validated against a primary standard, but for molecular testing this may not be possible. Sources of control materials may include commercially available organisms and nucleic acid samples; previously tested and stored patient specimens or their derivatives; inter-laboratory exchanges; materials distributed by proficiency testing surveys; or publicly available collections such as the American Type Culture Collection (www.atcc.org) and the Coriell Cell Repositories (http://ccr.coriell.org).

What Programs Exist to Provide Human Mutation Samples or Synthetic Samples for Use as Controls?

Published studies of proficiency programs are summarized in Table 6. Of the materials distributed, nine used actual patient specimens; 14 used cultured bacteria or viruses; four used cell cultures; one used cultured cells infected with viruses; and seven used artificial samples (plasmids or patient specimens spiked with the target materials). These numbers do not add up to 33 because some proficiency testing programs distributed more than one type of material.

Table 6. Sources of Control Materials in Published Proficiency Testing Programs

Source	Number of Publications Identified	References
CAP	4	112-114,137
EMQN	1	142
ENVID	1	122
EQAP	2	138,139
EQUAL	9	115,116,126-130,140,141
INQAT	2	117,119
NEQUAS	2	111,121
Reference laboratories	12	118,120,123-125,131-136,143

CAP College of American Pathologists
EMQN European Molecular Genetics Quality Network
ENVID European Network for Diagnostics of Imported Viral Diseases
EQAP Spanish external quality assessment program
EQUAL European Union Quality Control Concerted Action (now referred to as the Quality Control for Molecular Diagnostics, or QCMD)
INQAT Italian Network for Quality Assurance of Tumor Biomarkers
NEQAS National Quality Assessment Scheme (UK)

The Centers for Disease Control (CDC) organized two workshops in 2003 and 2004 to discuss needs for control materials and to develop recommendations to fulfill these needs.[23] As a consequence of these workshops, the CDC established the Genetic Testing Reference Materials Coordination Program (Ge T-RM), formerly known as the Genetic Testing Quality Controls Materials Program (GTQC) (http://wwwn.cdc.gov/dls/genetics/rmmaterials/default.aspx). This repository currently has only a limited number of control materials available, but plans to gradually expand in scope. The CDC is also funding efforts to develop synthetic samples that can be used in place of actual patient specimens.[144]

In addition, Qnostics currently sells proficiency testing material used in previous Quality Control for Molecular Diagnostics (QCMD) proficiency testing surveys (www.qnostics.com/QCMDPanels.htm).

To What Extent do Laboratories Exchange Samples Voluntarily for the Purpose of Proficiency Testing?

Our search of peer-reviewed journals and gray literature sources did not identify any studies (e.g., surveys) that provide sufficient information for us to make an estimation of the extent to which laboratories exchange samples voluntarily for PTs.

What Method-specific Proficiency Tests Have Been Developed?

The characteristics of the proficiency testing programs in the 33 published studies we identified are summarized in Table 7. The majority of the programs were intended to test the ability of the laboratory to identify or quantify a particular target gene or organism, using their own ASRs and laboratory-developed testing protocols. Some of the general-method programs did provide analyte-specific reagents (PCR primers) as part of the proficiency test.

Table 7. Published Proficiency Testing Programs

Type of Program	Number of Publications Identified	References
FISH, general	1	114
FISH, specific target	3	111-113
FISH, analyte-specific	0	
PCR, general	6	115-120
PCR, specific target	19	121-139
PCR, analyte-specific	0	
DNA sequencing, general	3	140-142
DNA sequencing, specific target	1	143
DNA sequencing, analyte-specific	0	

What Organizations or Programs are Implementing Proficiency Testing Programs for Molecular Tests, and What Standards have these Programs Established?

Under CLIA, each laboratory performing nonwaived testing must enroll in one CMS-approved proficiency testing (PT) program for each specialty, subspecialty, and analyte specified in Subpart I of the CLIA regulations (refer to Chapter 4 of the report). For tests that are not associated with any specialty, subspecialty, and analyte specified in Subpart I (e.g., molecular tests), laboratories are still required to participate in some equivalent activity such as exchanges of materials with other laboratories. Refer to the relevant sections in Chapter 5 and the CLIA regulations (42 CFR 493.1236(c)) for additional information.

In a recent overview of laboratory proficiency testing prepared for the Centers for Disease Control and Prevention, Peterson et al. recommended developing methods-based proficiency testing programs, rather than specific test-based proficiency testing programs, as the most practical way to address proficiency testing for molecular tests.[145]

In their recent report, the Secretary's Advisory Committee on Genetic, Health, and Society (SACGHS) recommended that studies of the effectiveness of other types of performance assessment methods be conducted to determine whether they are as robust as proficiency testing.[3]

College of American Pathologists

The College of American Pathologists (CAP) and the American College of Medical Genetics (ACMG) jointly administer a proficiency testing scheme (www.cap.org). In 2005, 285 laboratories within the U.S. and 52 from other countries participated in the program. Samples are sent out twice a year with three to five specimens per test in each shipment. The majority of the 17 currently available molecular tests involve testing for germline mutations (important for the pediatric population or prospective parents), which are beyond the scope of this Horizon Scan. The specimens typically consist of purified DNA or metaphase slide preparations, which omits the steps necessary for evaluating a laboratory's sample preparation competency. Reports are returned on a standardized form instead of in the laboratory's usual report format.

A laboratory that correctly tests 80% or more of the samples is graded as "acceptable." Laboratories that fail to achieve a satisfactory performance on three or four successive tests may be required to discontinue testing until the problem is corrected and verified to be corrected by successful completion of an external proficiency test.

CLIA-exempt States

Laboratories performing testing on New York State citizens are required to establish their proficiency twice a year (www.wadsworth.org/labcert/clep/clep.html). For tests with no available New York State proficiency test (e.g., most molecular tests) the laboratory itself is required to set up an internal proficiency testing program that may include split-sample performance compared to another validated method, evaluation of clinical outcomes, blind testing of specimens with known results, or other equivalent system. In 2008, CLEP planned to send out several virology proficiency testing panels that may be applicable to molecular diagnostics (www.wadsworth.org/labcert/clep/PT/PTschedule.htm).

Washington State does not itself provide a proficiency testing program, rather, they require proficiency testing be performed as specified by CLIA, and use the proficiency testing programs organized by CAP for monitoring molecular tests (www.doh.wa.gov/hsqa/FSL/lqa_PT_providers.htm).

Programs in Europe

Quality Control for Molecular Diagnostics

Quality Control for Molecular Diagnostics (QCMD), previously known as the European Union Quality Control Concerted Action (EQUAL), designs and develops quality control materials and proficiency testing programs. In 2008, they offered 29 proficiency testing panels for various infectious diseases (www.qcmd.org). Summary reports from all of their past proficiency tests are available at http://www.qcmd.org/Index2.htm.

European Molecular Genetics Quality Network

The European Molecular Genetics Quality Network (EMQN) also operates proficiency testing schemes in Europe (www.emqn.org). The program is voluntary and any laboratory may register and participate. Samples are distributed annually. In 2007, 18 disease-specific proficiency tests and two technique-specific tests (mutation scanning and DNA sequencing) were offered. The majority of the disease-specific tests involve testing the pediatric population or prospective parents for germline mutations. For each test, three samples of purified DNA set

within the context of a mock clinical case are sent to each participating laboratory. Laboratory reports are returned to the organizer. Laboratories that are scored as poor performers are contacted informally and offered advice on how to improve performance.

National Quality Assessment Scheme

The National Quality Assessment Scheme (NEQAS) for molecular genetics operates proficiency testing programs in the United Kingdom, the Netherlands, and Ireland (www.ukneqas.org.uk). Approximately 40 laboratories participate in the testing scheme each year. The intent of the program is to assess not only the proficiency of the laboratories in genotyping, but also their ability to interpret the results in the context of a realistic clinical scenario. The main function of the organizer is to inform participating laboratories when their performance falls below acceptable levels, and to offer advice and assistance to ensure the laboratory's performance returns to an acceptable level. NEQAS currently offers testing material for 15 different tests. Some tests involve testing the pediatric population or prospective parents for germline mutations and others are for adult onset heritable conditions, infectious disease, and HLA typing. NEQAS has recently begun offering proficiency testing for FISH assays for *ERBB2* (i.e., *HER-2/neu*) status in breast cancer;[111] determination of *ERBB2* status in breast cancer may be important for clinical decisions about treatment.

Samples (generally purified DNA) are distributed annually. For each disease, each participating laboratory receives three samples set within the context of a realistic clinical scenario. Performance levels are scored for each individual disease, rather than on an average score across all disease areas. A single significant genotyping error is enough to result in the designation of poor performance. If the laboratory report contains advice which is considered to be dangerously erroneous, the laboratory will also be scored as a poor performer. Non-participation in any available test is also scored as poor performance. Persistent poor performance is defined as poor performance for a particular disease in two consecutive years or poor performance in three out of six years.

European Network for Diagnostics of Imported Viral Diseases

The European Network for Diagnostics of Imported Viral Diseases (ENVID) is an organization devoted to diagnostics of "imported," rare and emerging viral infections of European interest. They organize limited proficiency testing programs and encourage exchange of testing materials between laboratories and the development of standardized testing methods (www.enivd.de/manifest.htm).

Italian Network for Quality Assurance of Tumor Biomarkers

The Italian Network for Quality Assurance of Tumor Biomarkers (INQAT) operates proficiency testing programs for tumor biomarkers in Italy (http://www.oncologico.bari.it/).

Program in Australia

The Human Genetics Society of Australia coordinates a proficiency testing program for molecular genetics (www.hgsa.com.au/Index.cfm?pid=111713).

What Proficiency Test Results for Molecular Testing have been Published by the Identified Testing Organizations?

The published results of proficiency testing performed by formal proficiency testing organizations are briefly summarized in Table 8. For full details of the reported results, refer to Table 33 and Table 34 in Appendix C.

Table 8. Published Proficiency Test Results of Formal Testing Programs

Organization	Test Program	Number of Surveys Performed	Results	References
CAP	FISH to detect amplification of *ERBB2* (i.e., *HER-2/neu*)	5	94 to 100% of all results were correct	112,113
	FISH in general	7	85 to 100% of all results were correct	114
	PCR-based detection of genetic alterations relevant to oncology	8	91 to 94% of all results were correct	137
EMQN	DNA sequencing	1	59% of laboratories were rated "excellent"	142
ENVID	PCR-based detection and typing of viruses	1	80 to 85% of all results were correct	122
EQAP	PCR-based detection of genetic alterations relevant to oncology	7	90% of all results were correct	138,139
EQUAL	PCR in general	2	73 to 80% of laboratories performed acceptably	115,116
	PCR-based detection and typing of viruses	8	28 to 98% of all results were correct	126-130
	DNA sequencing	2	There was considerable variation in the returned results. A training program resulted in a significant improvement in proficiency	140,141
INQAT	PCR in general	2	44 to 61% of laboratories were rated as "excellent" or "good"	117,119
NEQAS	FISH to detect amplification of *ERBB2 (HER-2/neu*)	1	60% of all results were "acceptable"	111
	PCR-based detection and typing of viruses	1	87 to 100% of all results were correct	121

CAP College of American Pathologists
EMQN European Molecular Genetics Quality Network
ENVID European Network for Diagnostics of Imported Viral Diseases
EQAP Spanish external quality assessment program
EQUAL European Union Quality Control Concerted Action (now referred to as the Quality Control for Molecular Diagnostics, or QCMD)
INQAT Italian Network for Quality Assurance of Tumor Biomarkers
NEQAS National Quality Assessment Scheme (UK)

What Factors have been Identified Which Contribute to Variability Within and Across Laboratories Performing Molecular Testing?

Factors reported to contribute (or not contribute) to molecular testing performance variability between and within laboratories are summarized in Table 9. Note that many studies did not explicitly define factors they had examined that were found not to contribute to such variability. Most of the published studies of proficiency testing reported that they were unable to determine causes of variability in the results.

The factor most commonly reported to have caused variability in results was the use of testing material that had very low levels of the target (i.e., genetic analytes of interest). FISH testing programs reported that the use of a low-level amplification sample caused close to 100% of errors.[111,112]

Many of the authors of the published proficiency test programs commented that although laboratories used a wide variety of methods, most were able to correctly perform the proficiency tests. The overall performance of the laboratory was not dependent on the methodology chosen; high-quality results instead seem to be dependent on the laboratory's internal quality control and optimization of the assay used.

Table 9. Reported Factors that Contributed to Molecular Testing Variability

Factor	Number of Studies Reporting it Contributed to Variability in Results	Number of Studies Reporting it Reduced Variability in Results	Number of Studies Reporting it Did Not Affect the Variability of Results
Use of commercial assay kits	1 [128]	3 [122,124,135]	1 [125]
Use of laboratory-developed assays	2 [122,124]	1 [128]	1 [125]
Use of commercial DNA preparation kits	1 [120]	0	1 [122]
Use of laboratory-developed DNA preparation methods	0	1 [120]	0
Samples with very low levels of the test target	12 [111,112,121,123,125-128,135,136,138,139]	0	0
Laboratory experience with the test	0	1 [141]	3 [114,118,143]
Laboratory had a good quality control plan in place	0	2 [114,123]	0
Staff proficiency in testing	0	1 [129]	0
Use of non-optimal DNA extraction methods	2 [115,119]	0	0
Use of non-optimal reagents	3 [115,119,120]	0	0
Use of non-optimal PCR parameters	5 [115,119,120,122,139]	0	0
Use of different PCR primers	2 [131,133]	0	5 [123,129,132,133,137]
Use of different PCR platforms	1 [117]	0	6 [116,123,129,132,133,137]

Do FDA-reviewed Tests Perform Better than Laboratory-developed Tests for the Same Clinical Intended Use?

Two of the published studies reported that the use of FDA-approved commercially available tests reduced variability in the results,[122,124] but one reported that the use of FDA-approved tests actually increased variability in comparison to the use of LDMTs,[128] and one reported that it did not affect the results.[125] The authors of one study commented that many of the commercially available FDA-approved tests were not sensitive enough to detect clinically relevant but low viral copy levels that many of the in-house assays were able to detect.[128] Braun et al. reported that the quality of DNA prepared using commercially available kits was, on average, lower than the DNA prepared by in-house-developed methods.[120]

Extent of Analytical Validation of the Test

None of the published studies provided information about the extent and quality of analytical validation of the tests being used by the participating laboratories. Authors of nine of the published studies speculated that the use of non-optimal testing methods by some laboratories may have contributed to the variability across laboratories (see Table 9).

Test Methodology

In general, proficiency testing demonstrated that FISH tests were rarely performed incorrectly (Table 8). Results for PCR-based tests varied across proficiency testing programs. Proficiency tests that required laboratories to extract DNA before performing PCR generally reported more variation in results than test programs that provided purified DNA.

Personnel

None of the published proficiency testing programs commented on personnel employed by the participating laboratories.

McGovern et al. surveyed 133 laboratories and assigned quality scores based on how the laboratory scored on a checklist of common laboratory practices thought to contribute to high quality performance.[146] They reported that higher quality scores were associated with the directors of the laboratories having a MD degree vs. a PhD degree, and higher quality scores were also associated with the laboratory directors being board-certified in biochemical genetics.

Environmental Conditions

None of the published proficiency testing programs commented on environmental conditions (temperature, air quality, etc.) in the participating laboratories.

Based on Published Studies, What Impact have these Proficiency Testing Programs had on Reducing Variation Within and Between Laboratories?

We identified several published studies that addressed the impact of proficiency testing on reducing variability of testing. In one study, Niedrig and colleagues reported that the proficiency of 33 laboratories in using PCR to detect and identify viruses was not significantly improved since the first survey conducted by the European Network for Diagnostics of Imported Viral Diseases (ENIVD).[122] In another study, Mascarello et al. reported that the proficiency of approximately 130 laboratories in performing FISH tests improved from 1997 to 2000, and the CTLN reported that the proficiency of 12 laboratories in performing PCR tests improved from 2000 to 2002, but it is unclear if the improvements were due to repeated proficiency tests, improvements in technology, or simply greater experience on the part of the laboratories in performing the tests.[114,134] Wang et al. reported that the accuracy of the laboratories improved over the five years after establishment of the proficiency testing program, but the authors of the report attributed the improvement to the development of higher-quality commercial testing kits, not the proficiency testing program.[135] Other published studies reported no significant changes in laboratory proficiency upon repeated proficiency testing.[113,126-128]

As part of the EQUAL program, Dorn-Beineke reported the impact of a training program on proficiency. Eight laboratories that had performed poorly in a DNA sequencing proficiency

test were invited to participate in a three-day training program. Six of the eight participated, and their proficiency scores significantly improved.[140]

What Data are Available from the Accrediting Organizations on the Impact of Proficiency Testing Programs on Laboratory Performance?

NEQAS reports that poor performance on their proficiency tests is a sporadic event with a low incidence of recurrence. Identified causes of errors include accidental sample swapping, clerical or interpretive errors, and problems with test analytic validity. Since 1997, NEQAS has identified a single incident of persistent poor performance. The laboratory's analytical errors were reviewed and corrected to the satisfaction of all concerned.[147]

Chapter 8. What Guidelines and Standards Exist for Laboratories Conducting Molecular Testing?

For Key Question 8, we were asked to identify guidelines and standards for laboratories conducting molecular testing.

Methods

To begin identifying guidelines and standards, we searched the Web sites of the following organizations listed in the statement of work: American College of Medical Genetics, College of American Pathologists, American Society for Clinical Oncology, National Institute of Standards and Technology, Clinical and Laboratory Standards Institute, and the New York State Clinical Laboratory Evaluation Program. We also searched the National Guideline Clearinghouse™ and ECRI Institute's Healthcare Standards database for additional clinical practice guidelines, published standards, position papers, and peer-reviewed literature.

Results

Our searches identified a total of 34 guidelines and standards for laboratories conducting molecular tests from the above-mentioned organizations. Twenty-five of the 34 guidelines and standards are sponsored by organizations from the United States, with the remaining ones sponsored by European or Australian organizations. The vast majority of the identified guidelines and standards were published within the past five years. The Clinical Laboratory Standards Institute (CLSI), a U.S.-based organization that develops laboratory standards based on voluntary consensus, is the most prominent sponsor of molecular-test-related guidelines. The 12 guidelines published by CLSI cover a variety of technical issues regarding different types of molecular methods. Table 10 provides a summary of the 41 guidelines and standards. The table highlights the key technical areas covered by the guidelines or standards, including: testing techniques, testing samples, testing validation and verification, proficiency testing, sensitivity and specificity, quality control and quality assurance, safety, results interpretation and reporting, clinical utility, laboratory, personnel, and clinicians, accreditation, certification, and program evaluation, test regulation and recommendations. In addition, we identified 38 peer-reviewed articles that describe or discuss guidelines or standards relevant to molecular testing.

For detailed information about each standard or guideline, including its publisher, title, purposes, description, year of publication, and reference number, refer to Table 36 in Appendix E. Note that, although the U.S. Food and Drug Administration (FDA) has published a series of guidance documents relevant to molecular testing, we only include the two documents that are most relevant to laboratory-developed tests in this chapter. For other FDA-issued guidances, refer to Chapter 5.

Table 10. Summary of Guidelines and Standards for Laboratories Performing Molecular Tests

Source	Title	Year	Region	Testing Techniques	Testing Samples	Testing Validation and Verification	Proficiency Testing	Sensitivity and Specificity	Quality Control and Quality Assurance	Safety	Results Interpretation and Reporting	Clinical Utility	Laboratory, Personnel, and Clinician	Accreditation, Certification, and Program Evaluation	Test Regulations and Recommendations	Reference
NACB	Guidelines and Recommen-dations for Laboratory Analysis and Application of Pharma-cogenetics to Clinical Practice	2006	USA	X					X		X	X			X	[148]
FDA	Guidance for Industry and FDA Staff Commercially Distributed Analyte Specific Reagents (ASRs): Frequently Asked Questions	2007	USA												X	[33]

Source	Title	Year	Region	Testing Techniques	Testing Samples	Testing Validation and Verification	Proficiency Testing	Sensitivity and Specificity	Quality Control and Quality Assurance	Safety	Results Interpretation and Reporting	Clinical Utility	Laboratory, Personnel, and Clinician	Accreditation, Certification, and Program Evaluation	Test Regulations and Recommendations	Reference
FDA	Draft Guidance for Industry, Clinical Laboratories, and FDA Staff - In Vitro Diagnostic Multivariate Index Assays (IVDMIAs)	2007	USA												X	33
CLSI	Molecular Diagnostic Methods for Infectious Disease; Approved Guideline 2nd Edition	2006	USA	X					X		X				X	149
CLSI	Molecular Diagnostic Methods for Genetic Diseases; Approved Guideline 2nd Edition	2006	USA	X		X			X	X	X					13

Source	Title	Year	Region	Testing Techniques	Testing Samples	Testing Validation and Verification	Proficiency Testing	Sensitivity and Specificity	Quality Control and Quality Assurance	Safety	Results Interpretation and Reporting	Clinical Utility	Laboratory, Personnel, and Clinician	Accreditation, Certification, and Program Evaluation	Test Regulations and Recommendations	Reference
CLSI	Genotyping for Infectious Diseases: Identification and Characteriza-tion; Approved Guideline	2006	USA	X		X					X	X				150
ACTG	Virology Quality Assessment (VQA) Program Testing Requirements for New Laboratories to Obtain Approval for HIV DNA Testing in NIH-Funded Protocols	2004	USA											X		151
CMGS	Practice Guidelines for Internal Quality Control within the Molecular Genetics Laboratory	2004	United Kingdom	X	X				X							152

Source	Title	Year	Region	Testing Techniques	Testing Samples	Testing Validation and Verification	Proficiency Testing	Sensitivity and Specificity	Quality Control and Quality Assurance	Safety	Results Interpretation and Reporting	Clinical Utility	Laboratory, Personnel, and Clinician	Accreditation, Certification, and Program Evaluation	Test Regulations and Recommendations	Reference
MIAME	The MIAME Checklist	2005									X					153
New York State Depart-ment of Health - Wads-worth Center	Clinical Laboratory Evaluation Program (CLEP) - Guide to Program Requirements and Services	2005	USA	X	X	X	X	X	X	X	X		X	X	X	154
NSW Depart-ment of Health	Genetic Testing - Guidelines for Prioritizing Genetic Tests	2007	Australia				X									155
CLSI	Quantitative Molecular Methods for Infectious Diseases	2003	USA	X	X	X	X				X	X				156

Source	Title	Year	Region	Testing Techniques	Testing Samples	Testing Validation and Verification	Proficiency Testing	Sensitivity and Specificity	Quality Control and Quality Assurance	Safety	Results Interpretation and Reporting	Clinical Utility	Laboratory, Personnel, and Clinician	Accreditation, Certification, and Program Evaluation	Test Regulations and Recommendations	Reference
CLSI	Proficiency Testing (External Quality Assessment) for Molecular Methods; Approved Guideline	2005	USA										X			157
CLSI	Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline	2005	USA	X						X						158
CLSI	Use of External RNA Controls in Gene Expression Assays; Approved Guideline	2006	USA			X			X		X					159

Source	Title	Year	Region	Testing Techniques	Testing Samples	Testing Validation and Verification	Proficiency Testing	Sensitivity and Specificity	Quality Control and Quality Assurance	Safety	Results Interpretation and Reporting	Clinical Utility	Laboratory, Personnel, and Clinician	Accreditation, Certification, and Program Evaluation	Test Regulations and Recommendations	Reference
CLSI	Verification and Validation of Multiplex Nucleic Acid Assays; Proposed Guideline	2007	USA	X	X	X					X					15
CDC/ MMWR	Guidelines for Laboratory Test Result Reporting of Human Immuno-deficiency Virus Type 1 Ribonucleic Acid Determination	2001	USA								X					160
CDC/ MMWR	Good Laboratory Practices for Waived Testing Sites	2005	USA				X		X	X					X	161

Source	Title	Year	Region	Testing Techniques	Testing Samples	Testing Validation and Verification	Proficiency Testing	Sensitivity and Specificity	Quality Control and Quality Assurance	Safety	Results Interpretation and Reporting	Clinical Utility	Laboratory, Personnel, and Clinician	Accreditation, Certification, and Program Evaluation	Test Regulations and Recommendations	Reference
CDC/MMWR	Good Laboratory Practices for Molecular Genetic Testing for Heritable Diseases and Conditions	2009	USA	X	X	X	X	X	X		X		X		X	[4]
ACMG	Standards and Guidelines for Clinical Genetics Laboratories Technical Standards & Guidelines: Molecular Genetic Testing for Ultra-Rare Disorders	2006	USA	X		X					X					[162]
CAP	Laboratory Accreditation Program - Molecular Pathology Checklist	2007	USA	X	X	X	X	X	X	X	X		X	X		[163]

Source	Title	Year	Region	Testing Techniques	Testing Samples	Testing Validation and Verification	Proficiency Testing	Sensitivity and Specificity	Quality Control and Quality Assurance	Safety	Results Interpretation and Reporting	Clinical Utility	Laboratory, Personnel, and Clinician	Accreditation, Certification, and Program Evaluation	Test Regulations and Recommendations	Reference
CAP	Clinical Laboratory Reports in Molecular Pathology	2007	USA								X					[36]
CAP	Recom-mended Principles and Practices for Validating Clinical Molecular Pathology Tests	2009	USA			X		X			X				X	[7]
ACMG	Standards and Guidelines for Clinical Genetics Laboratories	2006	USA	X	X	X			X							[8]
European Com-mission	Guidance Document on the Use of Reference Materials in Genetic Testing	2008	Europe	X		X			X							[164]

Source	Title	Year	Region	Testing Techniques	Testing Samples	Testing Validation and Verification	Proficiency Testing	Sensitivity and Specificity	Quality Control and Quality Assurance	Safety	Results Interpretation and Reporting	Clinical Utility	Laboratory, Personnel, and Clinician	Accreditation, Certification, and Program Evaluation	Test Regulations and Recommendations	Reference
European co-operation for Accredita-tion	Use of Proficiency Testing as a Tool for Accreditation testing	2001	Europe										X			165
Standards Unit, Evalua-tions and Standards Labora-tory	Good Laboratory Practice When Performing Molecular Amplification Assays	2006	Europe				X									35
European Com-mission	25 Recommenda-tions on the ethical, legal, and social implications of genetic testing	2004	Europe							X					X	166
Swiss Society of Medical Genetics	Best Practice Guidelines on Reporting in Molecular Genetic Diagnostic Laboratories in Switzerland	2003	Europe								X					167

Source	Title	Year	Region	Testing Techniques	Testing Samples	Testing Validation and Verification	Proficiency Testing	Sensitivity and Specificity	Quality Control and Quality Assurance	Safety	Results Interpretation and Reporting	Clinical Utility	Laboratory, Personnel, and Clinician	Accreditation, Certification, and Program Evaluation	Test Regulations and Recommendations	Reference
DynCorp Health Research Services	General Recommenda-tions for Quality Assurance Programs for Laboratory Molecular Genetic Tests	1999	USA						X				X			168
EMQN	Draft Best Practice Guidelines for Laboratory Internal Quality Control	2002	Europe		X	X					X					34
OECD	Guidelines for Quality Assurance in Molecular Genetic Testing	2007	Europe						X							169
CLSI	Immuno-globulin and T-cell Receptor Gene Rearrange-ment Assays; Approved Guideline	2002	USA	X	X			X	X		X					170

Source	Title	Year	Region	Testing Techniques	Testing Samples	Testing Validation and Verification	Proficiency Testing	Sensitivity and Specificity	Quality Control and Quality Assurance	Safety	Results Interpretation and Reporting	Clinical Utility	Laboratory, Personnel, and Clinician	Accreditation, Certification, and Program Evaluation	Test Regulations and Recommendations	Reference
CLSI	Nucleic Acid Amplification Assays for Molecular Hemato-pathology; Approved Guideline	2003	USA		X			X	X		X					12
CLSI	Fluorescence *In Situ* Hybridization (FISH) Methods for Medical Genetics; Approved Guideline	2004	USA	X											X	14
CLSI	Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine; Approved Guideline	2004	USA		X						X					171
CLSI	Diagnostic Nucleic Acid Microarrays; Approved Guideline	2006	USA		X	X			X		X					11

Source	Title	Year	Region	Testing Techniques	Testing Samples	Testing Validation and Verification	Proficiency Testing	Sensitivity and Specificity	Quality Control and Quality Assurance	Safety	Results Interpretation and Reporting	Clinical Utility	Laboratory, Personnel, and Clinician	Accreditation, Certification, and Program Evaluation	Test Regulations and Recommendations	Reference
NHGRI	Promoting Safe and Effective Genetic Testing in the United States	1997	USA							X						172
ASCO	Recommenda-tions for the Use of Tumor Markers in Breast Cancer Update	2007	USA									X				173
NCCN	Breast Cancer Onco*type* DX assay	2009	USA									X				174
AMP	Recommenda-tions for In-House Development and Operation of Molecular Diagnostic Tests	1999	USA	X	X	X	X	X	X		X		X		X	175
Peer-reviewed articles				24,156	156,176	20,21,156,177,178	146,176,179,180		22,30,119,146,156,177,181-190	184	156	24	118,156,183,189,191	17,192-196	184,186,189, 197-203	

ACMG American College of Medical Genetics
ACTG AIDS Clinical Trials Group
AMP Association for Molecular Pathology Statement

ASCO	American Society of Clinical Oncology
CAP	College of American Pathologists
CDC/ MMWR	Centers for Disease Control and Prevention – Morbidity and Mortality Weekly Report
CLSI	Clinical Laboratory Standards Institute
CMGS	Clinical Molecular Genetics Society
EMQN	European Molecular Genetics Quality Network
FDA	Food and Drug Administration
MIAME	Minimum information about a microarray experiment
NACB	National Academy of Clinical Biochemistry
NCCN	National Comprehensive Cancer Network
NHGRI	National Human Genome Research Institute
NSW Department of Health	New South Wales Department of Health
OECD	Organisation for Economic Co-Operation and Development

Epilogue

Molecular testing is a rapidly expanding area of research and clinical application. The strong growth of this area has largely been fueled by the many groundbreaking advances in genetic research and molecular technology, such as the completion of the Human Genome Project. While the introduction of a wide array of molecular tests has the potential to improve quality of care, many stakeholders have voiced concern about the quality of these tests, in addition to concern about their safety, effectiveness, cost, and ethical implications.[3,89,204,205] Laboratory-developed molecular tests (LDMTs) are of particular concern because they comprise the majority of currently available molecular tests and they are not actively regulated by the FDA.

Various efforts have been initiated to address these concerns. The Secretary's Advisory Committee on Genetic, Health, and Society (SACGHS) was chartered in 2002 to assist the Secretary of Health and Human Services in evaluating policy issues arising from the diffusion of genetic testing into clinical and public health practice. Having identified oversight of genetic testing as a high priority issue, this committee developed a report on the subject and published the report in April 2008.[3] In this report, the SACGHS recommended funding for development of reference methods, materials and biological samples necessary for assuring the analytic validity of genetic tests. They also recommended additional research to develop robust methods for assessing the performance of the laboratories offering genetic tests other than the current proficiency testing model used by other laboratory disciplines.

In 2007, U.S. Senator Edward Kennedy and then-Senator Barack Obama introduced two proposals, respectively, to enhance genetic test oversight.[205] In July 2007, the FDA published a draft guidance indicating the Agency's intention to regulate in-vitro diagnostic multivariate index assays (IVDMIAs) as medical devices.[93] Under the proposed guidance, some LDMTs would be categorized as IVDMIAs. The State of New York, one of the two CLIA-exempt States, has already been using standards that are arguably more stringent than those used by other regulatory bodies to evaluate laboratory-developed tests. Under New York State's regulations, all laboratories performing molecular tests on specimens collected from the State are required to obtain pre-approval prior to offering a test.[3]

The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) project was initiated by CDC in 2005 to develop a rigorous, evidence-based process for evaluating genetic tests and their applications for clinical and public health practice. The EGAPP Working Group published a paper in 2008, describing the methods that it developed for establishing the evidence-based assessment process.[2] In June 2009, the Clinical Laboratory Improvement Advisory Committee (CLIAC) Genetic Testing Good Laboratory Practices Workgroup published a report that made recommendations for good laboratory practices for ensuring the quality of molecular genetic testing.[4] 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.

Our report is among these efforts to address the concerns about the quality of LDMTs. Each of the chapters in our report focuses on one Key Question. In Chapter 1, we addressed

Key Question 1 with the goal of providing a quick overview of the molecular tests (both laboratory-developed tests and commercially available kits) currently available to the Medicare over-65-year-old population. Our searches identified 1,441 such tests—of which 812 were clearly identified as laboratory-developed tests—performed by 95 laboratories. Given the time frame of this project, we used the AMP Web site as our primary source for identifying LDMTs of interest. The AMP Web site was chosen over other sites of similar purpose (e.g., www.genetests.org) because the AMP provided more information on the tests and the laboratories. This information permitted us to determine whether the tests within the scope of this Horizon Scan. We also identified a small number of LDMTs from non-AMP sources (e.g., literature reviews and the Web sites of commercial laboratories).

In Chapters 2, 3, 4 and 7, we addressed the Key Questions concerning how analytic validity, clinical validity and clinical utility are established for molecular tests. Our approach to addressing these questions is primarily based on literature review, with published systematic reviews given the highest priority. This approach allowed us to shed light on some of the more popular molecular tests currently in clinical use. The identified systematic reviews suggest that validation of molecular tests is challenging due to some technical hurdles, such as lack of "gold-standard" reference methods and difficulty to obtain testing samples. As a result, the analytic and clinical validity of these tests was often not backed up by quality data in the public domain. Without analytic and clinical validity of molecular tests being adequately established and due to lack of studies—particularly RCTs—that correlate test results with clinical outcomes, clinical utility of molecular tests is difficult to establish.

In Chapters 4, 5, and 6, we addressed the Key Questions regarding the oversight of molecular tests. In addressing these questions, we reviewed government documentations for first-hand information. We also interviewed key government officials from relevant regulatory bodies to collect or confirm information. The information that we collected reveals that, although the FDA claims the authority to oversee laboratory-developed tests, including LDMTs, the agency has been exercising enforcement discretion by far. However, there is a possibility that the agency may step up its oversight on a subset of LDMTs that falls into the category of in vitro diagnostic multivariate index assays (IVDMIAs). The agency has published a draft guidance for industry, clinical laboratories, and the FDA staff, signaling that IVDMIAs might be under the agency's oversight in the future (discussed in Chapter 5).

Under the Clinical Laboratory Improvement Amendments (CLIA) program, laboratories are required to establish the analytic validity of LDMTs if they perform such tests. However, due to the reasons previously discussed, establishing the analytic validity of LDMTs is a challenging task. Meanwhile, there are concerns about whether the clinical validity and utility of LDMTs have been addressed adequately, or at all, under CLIA.[3] Although CLIA requires laboratory directors and clinical consultants to ensure the clinical relevance of the tests being performed, how laboratory directors and clinical consultants establish the clinical relevance (e.g., what types of data were used, where the data came from, and how the data were synthesized) have rarely been revealed to the public.

In Chapter 8, we provided a list of standards and guidelines concerning quality assurance and evaluation of molecular tests. The 41 guidelines and standards that we identified are sponsored by organizations from U.S., Europe, or Australia, and cover a variety of technical issues regarding different types of molecular methods. The vast majority of these guidelines and standards were published within the past five years. The Clinical Laboratory Standards Institute

(CLSI), a U.S.-based organization that develops laboratory standards based on voluntary consensus, is the most prominent sponsor of molecular-test-related guidelines. A number of these guidelines, particularly those sponsored by the CLSI, the College of American Pathologists, the Clinical Molecular Genetics Society, DynCorp Health Research Services, the European Molecular Genetics Quality Network, the National Academy of Clinical Biochemistry, the New York State Department of Health (Wadsworth Center), the Organization for Economic Cooperation and Development, and the Standards Unit of the Evaluation and Standards Laboratory of the Health Protection Agency in the United Kingdom, directly address the measures necessary to assure policymakers, clinicians and the public of the analytic validity and proficient performance of laboratory-developed tests.

Given the dynamic nature of the molecular testing area, the assessments of the quality, regulation, or utility of LDMTs need to be frequently updated. One of the major challenges for those making decisions about the oversight of LDMTs will be to keep pace with the future developments in the area. Currently, the experience of the Clinical Laboratory Evaluation Program in New York State should certainly provide some valuable lessons in how the oversight of LDMTs might be accomplished and what resources would be necessary to do so on a national scale.

At this stage in the evolution of laboratory-developed tests, assessments of the appropriate clinical applications and development of evidence-based guidelines for using test results in decision making are only beginning to appear. The EGAPP program is making important contributions in this area, but with the rapid expansion of test offerings, guidance for the use of most laboratory-developed tests will lag far behind. Furthermore, in the absence of specific reimbursement codes for specific laboratory-developed and other molecular tests, it will be difficult to track practice patterns and to understand the impact of these tests on the patient outcomes and on the practice of medicine.

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Appendix A. Literature Search Strategies

A variety of approaches were used to identify relevant information for this report, including searches of peer-reviewed literature, gray literature, and Federal regulations.

Part I

This portion of the search report includes searches of bibliographic resources for key questions 2, 3, and 7. ECRI Institute's search strategies employ combinations of freetext keywords as well as controlled vocabulary terms including (but not limited to) the following concepts. The strategies presented below are in OVID syntax; the searches were simultaneously conducted across EMBASE, MEDLINE, and CINAHL. Parallel strategies based on MeSH headings and keywords were used to search the databases comprising the Cochrane Library.

Medical Subject Headings (MeSH), EMTREE, CINAHL and Keywords

Conventions:

OVID

$	=	truncation character (wildcard)
exp	=	"explodes" controlled vocabulary term (e.g., expands search to all more specific related terms in the vocabulary's hierarchy)
.de.	=	limit to controlled vocabulary heading
.fs.	=	floating subheading
.hw.	=	limit to heading word
.md.	=	type of methodology (PsycINFO)
.mp.	=	combined search fields (default if no fields are specified)
.pt.	=	publication type
.ti.	=	limit to title
.tw.	=	limit to title and abstract fields

PubMed

[mh]	=	MeSH heading
[majr]	=	MeSH heading designated as major topic
[pt]	=	publication type
[sb]	=	Subset of PubMed database (PreMEDLINE, Systematic, OldMEDLINE)
[sh]	=	MeSH subheading (qualifiers used in conjunction with MeSH headings)
[tiab]	=	keyword in title or abstract
[tw]	=	text word

Topic-specific Search Terms

Many controlled vocabulary terms and keywords were considered for inclusion in the search strategies. The following table contains an alphabetical listing of terms and keywords grouped by broad concepts. These are the terms and keywords that were actually included in the final search strategies.

Concept	Controlled Vocabulary	Keywords
Accuracy	accuracy diagnostic accuracy likelihood exp prediction and forecasting/ predictive value of tests receiver operating characteristic ROC curve sensitivity and specificity	Assay linearity False negative False positive Gold standard Hook effect Sensitivity Specificity True negative True positive
Clinical validity	follow-up/ incidence/ mortality/	Clinical relevan$ Clinical util$ Clinical valid$ Course$ Predict$ Prognos$

Concept	Controlled Vocabulary	Keywords
Diseases/targets searched for Key Question 3	acute lymphocytic leukemia/ Acute myelomonocytic leukemia/ Acute promyelocytic leukemia B cell lymphoma/ Exp boradatella pertussis/ Chronic myeloid leukemia Cytochrome P450/ cytomegalovirus/ epidermal growth factor receptor 2 Epstein Barr virus/ hepatitis b/ and viral load hepatitis c/ and genotyp$ herpes simplex virus/ exp HIV/ exp human immunodeficiency virus/ leukemia, lymphocytic, acute/ leukemia, myeloid/ leukemia, myelomonocytic, acute/ Mantle cell lymphoma methicillin resistant staphylococcus aureus/ methicillin resistance/ mycobacterium tuberculosis/ mycoplasma infections/ mycoplasma pneumonia/ Oncogene Neu parvovirus/ parvovirus infections/ promyelocytic leukemia/ receptor, erbb-2 exp simplexvirus/ staphylococcus aureus/ T cell lymphoma/ Warfarin and genotyp$	Acute lymphocytic leukemia Acute myelomonocytic leukemia B cell lymphoma bcl-2 translocation Bordatella pertussis Cytochrome P450 Cytomegalovirus Epstein Barr virus Hepatitis b Hepatitis c HER 2 oncogene Herpes simplex virus Human immunodeficiency virus Human parvovirus B19 Human parvo virus B19 Mantle cell lymphoma Methicillin resistant staphylococcus aureus Mycobacterium tuberculosis Mycoplasma pneumonia Myeloid leukemia Neu Oncogene Oncogene C Neu Oncogene Erb B2 Oncogene Her 2 T cell lymphoma Warfarin BCR-ABL BCRABL CBFB-MYH11 CCFBMYH11 CYP450 EBV Erbb2 Erbb 2 Erbb-2 ErbB-2 Her2 Neu Her 2 Neu HER2/neu Her-2 Neu HIV HTLV-I HTLV-II MRSA PML-RARA PMLRARA

Concept	Controlled Vocabulary	Keywords
Laboratory-developed tests		Assembled Home brew Home-brew In house In-house Laboratory developed Laboratory-developed LDT LDTs
Molecular techniques	DNA microarray exp gene amplification/ exp genetic techniques/ exp hybridization/ exp in situ hybridization/ microarray analysis exp microarray analysis/ molecular diagnostic techniques exp molecular probe/ exp molecular probe techniques/ exp nucleic acid amplification techniques/ exp nucleic acid hybridization/	
Proficiency testing	Laboratories/standards Proficiency testing laboratory	external quality assessment EQA proficiency
Repeatability	Observer variation	Interpret$ Interobserver Inter-observer Intraobserver Intra-observer Kappa Observer bias Observer variability Reader$ Reliab$ Repeatab$ Replicat$
Validity		validity

Electronic Database Searches

The following databases have been searched for relevant information for Key Questions 1, 2, 3, and 7.

Name	Date Limits	Platform/Provider
CINAHL	1982 through October 31, 2008	OVID
The Cochrane Central Register of Controlled Trials (CENTRAL)	Through 2008, Issue 4	www.thecochranelibrary.com
The Cochrane Database of Methodology Reviews (Methodology Reviews)	Through 2008, Issue 4	www.thecochranelibrary.com
The Cochrane Database of Systematic Reviews (Cochrane Reviews)	Through 2008, Issue 4	www.thecochranelibrary.com
Database of Abstracts of Reviews of Effects (DARE)	Through 2008, Issue 4	www.thecochranelibrary.com
EMBASE (Excerpta Medica)	1980 through October 31, 2008	OVID
MEDLINE	1960 through October 31, 2008	OVID

Key Question 2 – Analytic Validity

CINAHL/EMBASE/MEDLINE/PreMEDLINE

Human/English language

Set Number	Concept	Search Statement
1	Molecular testing - EMTREE	exp molecular probe/ or exp hybridization/ or exp molecular probe/ or exp gene amplification/ or (Microarray analysis or DNA microarray).de.
2	Molecular testing - MeSH	molecular diagnostic techniques.de. or exp molecular probe techniques/ or exp nucleic acid amplification techniques/ or exp nucleic acid hybridization/ or exp in situ hybridization/ or exp microarray analysis/
3	Combine sets	1 or 2
4	Limit by publication type	3 not ((letter or editorial or news or comment or case reports or note or conference paper or review).de. or (letter or editorial or news or comment or case reports or review).pt.)
5	Accuracy	4 and (exp prediction and forecasting/ or (predictive value of tests or receiver operating characteristic or ROC curve or sensitivity and specificity or accuracy or diagnostic accuracy or precision or likelihood).de. or ((false or true) adj (positive or negative)))
6	Validity	4 and validity.ti,ab.
7	Repeatability	4 and ((intraobserver or intra-observer or interobserver or inter-observer or interpret$ or kappa or observer bias or observer variability or reader$ or reader concordance or reliab$ or repeatab$ or replicat$).tw. or observer variation.de.)
8	Combine sets	or/5-7
9	Limit by study type	7 and (research synthesis or (systematic review or meta analysis or meta-analysis).de. or ((evidence base$ or methodol$ or systematic or quantitative$ or studies).mp. and (review.de. or review.pt.)))
10	Eliminate overlap	Remove duplicates from 8
11	Assay linearity	4 and (assay linearity or hook effect)
12	Combine sets	11 not 12

Key Question 3 – Clinical Validity

Note: In addition to terms related to clinical validity/utility, these search strategies include search statements geared to retrieve citations relevant to specific conditions and targets.

CINAHL/EMBASE/MEDLINE/PreMEDLINE

Human/English language

Set Number	Concept	Search Statement
1	Laboratory developed tests	(((home brew or home-brew or homebrew or in-house or inhouse or assembled) and laborator$) or (laborator$ adj developed))
2	Laboratory diagnosis	Exp laboratory diagnosis/ or (laborator$ and (diagnos$ or technique$ or procedure$))
3	Genetics	Exp nucleotide sequence/ or nucleotide sequence or DNA or RNA
4		Exp genetic marker/ or exp genetic markers/ or exp biological markers/ or ((genetic or biologic$) adj marker$)
5	Clinical validity	Clinical adj (relevan$ or util$ or valid$)
6	Combine sets	or/1-6
7	Diagnosis	6 and (exp diagnostic procedure/ or exp diagnosis)
8	Prognosis	6 and (exp incidence/ or exp mortality/ or exp follow up/ or prognos$ or predict$ or course$)
9	Therapy	6 and (exp therapy/ or therap$ or treatment$)
10	Combine sets	or/7-9
11	Limit by publication type	10 not ((letter or editorial or news or comment or case reports or note or conference paper or review).de. or (letter or editorial or news or comment or case reports or review).pt.)
12	Limit by study type	11 and (research synthesis or (systematic review or meta analysis or meta-analysis).de. or ((evidence base$ or methodol$ or systematic or quantitative$ or studies).mp. and (review.de. or review.pt.)))
13	Her2/Neu	12 and ((oncogene neu or epidermal growth factor receptor 2 or receptor, erbb-2).de. or (her$ adj2 neu) or erbb2 or erbb 2 or erbb-2 or (oncogene adj3 (her$ or neu$ or erb$))
14	Leukemia & lymphoma	12 and (exp leukemia/ or exp lymphoma/ or ((myelomonocytic or promyelocytic or myeloid or lymphocytic) adj2 (leukemia$) or ((b cell or t cell or mantle cell) adj2 lymphoma) or CBFBMYH11 or CBFB-MYH11 or PML-RARA or PMLRARA or bcl-2 translocation or HTLV-I or HTLV-II)
15	Bordatella pertussis	12 and (bordatella pertussis.de. or bordatella pertussis)
16	Cytochrome p450	12 and (Cytochrome p450.de. or Cytochrome p450 or cyp450)
17	Cytomegalovirus	12 and (exp cytomegalovirus/ or cytomegalovirus or CMV)
18	Epstein Barr virus	12 and ((Epstein barr virus or Epstein barr virus infections).de. or EBV)
19	Hepatitis B	12 and (hepatitis b.de. and viral load)
20	Hepatitis C	12 and (hepatitis c.de. and geneotyp$)

Set Number	Concept	Search Statement
21	Herpes Simplex virus	12 and (exp herpes simplex virus/ or exp simplexvirus/)
22	Human immunodeficiency virus	12 and (exp human immunodeficiency virus/ or exp HIV/ or HIV$ or human immunodeficiency virus)
23	Mycobacterium tuberculosis	12 and (mycobacterium tuberculosis).mp.
24	Mycoplasma pneumonia	12 and (exp mycoplasma infections/ or mycoplasma pneumonia.mp.)
25	Methicillin-resistant staph aureus	12 and (methicillin resistant staphylococcus aureus.de. or (exp staphylococcus aureus/ and methicillin resistance.de.) or methicillin resistant staphylococcus aureus or MRSA)
26	Parvovirus	12 and (parvovirus or exp parvovirus infections/ or human parvovirus B19 or human parvo virus b19)
27	Warfarin monitoring	12 and (warfarin.mp. and genotyp$)
28	Combine sets	or/13-27

Key Question 7 – Proficiency Testing

CINAHL/EMBASE/MEDLINE/PreMEDLINE

Human/English language

Set Number	Concept	Search Statement
1	Molecular testing - EMTREE	exp molecular probe/ or exp hybridization/ or exp molecular probe/ or exp gene amplification/ or (Microarray analysis or DNA microarray).de.
2	Molecular testing - MeSH	molecular diagnostic techniques.de. or exp molecular probe techniques/ or exp nucleic acid amplification techniques/ or exp nucleic acid hybridization/ or exp in situ hybridization/ or exp microarray analysis/
3	Combine sets	1 or 2
4	Limit by publication type	3 not ((letter or editorial or news or comment or case reports or note or conference paper or review).de. or (letter or editorial or news or comment or case reports or review).pt.)
5	Proficiency testing	4 and (proficiency testing laboratory.de. or proficiency$.ti. or external quality assessment or EQA)
6	Limit by publication type	5 and (research synthesis or (systematic review or meta analysis or meta-analysis).de. or ((evidence base$ or methodol$ or systematic or quantitative$ or studies).mp. and (review.de. or review.pt.)))
7	Eliminate overlap	Remove duplicates from 6

Part 2

The following databases have been searched for relevant information for Key Questions 4, 5, 6 and 8.

Name	Date Limits	Platform/Provider
Federal Register	Scanned throughout project	http://www.gpoaccess.gov/fr/
Healthcare Standards	Searched November 26, 2008	www.ecri.org
Lexis-Nexis Congressional bill tracking	Searched March 4, 2008	www.lexis.com
Lexis-Nexis Major Newspapers	Searched March 6, 2008	www.lexis.com
National Guideline Clearinghouse™ (NGC)	Searched November 26, 2008	www.guideline.gov

The following Web sites have been mined for information relevant to Key Questions 4, 5, 6 and 8.

Name	URL
American Association for Laboratory Accreditation	www.a2la.org
American College of Medical Genetics (ACMG)	www.acmg.net
American Society of Clinical Oncology (ASCO)	www.asco.org
Association for Molecular Pathology	www.amp.org
CanGeneTest.org	http://www.cangenetest.org/en/index.html
Centers for Disease Control and Prevention (CDC) • CLIAC • External Quality Assessment • Genetic testing	www.cdc.gov http://wwwn.cdc.gov/cliac/ http://wwwn.cdc.gov/mlp/eqa.aspx wwwn.cdc.gov/dls/genetics/default.aspx
Centers for Medicare and Medicaid Services (CMS)	www.cms.gov
Clinical and Laboratory Standards Institute (CLSI) National Committee of Clinical Laboratory Standards (NCCLS)	http://www.clsi.org/
College of American Pathologists (CAP)	www.cap.org
EuroGentest	www.eurogentest.org/
Eurolab	http://www.eurolab.org/
European Molecular Genetics Quality Network	http://www.emqn.org/emqn/

Name	URL
Food and Drug Administration (FDA) • CFR – clinical chemistry & toxicology devices • Genomics • Guidance - IVDMIA • Office of In Vitro Diagnostics • 510(k) database	www.fda.gov http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?CFRPart=862 www.fda.gov/cder/genomicss/ http://www.fda.gov/ohrms/dockets/dockets/06d0347/06d0347.htm http://www.fda.gov/cdrh/oivd/
Genetics and Public Policy Center	http://www.dnapolicy.org/
International Accreditation Forum (IAF)	www.iaf.nu
International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)	www.ifcc.org
International Laboratory Accreditation Collaboration	www.ilac.org
Joint Commission • Proficiency testing	www.jointcommission.org http://www.jointcommission.org/AccreditationPrograms/LaboratoryServices/ProficiencyTesting/default.htm
National Association of Testing Authorities (NATA)	http://www.nata.asn.au/
National Health Service	www.nhs.gov
National Human Genome Research Institute	www.genome.gov
National Institute of Standards and Technology (NIST)	www.nist.gov
New Zealand Accreditation Authority	www.ianz.govt.nz/
Organisation for Economic Co-operation and Development (OECD)	www.oecd.org
Pharmacogenetics & Pharmacogenomics Knowledgebase	http://www.pharmgkb.org/
PHG Foundation	http://www.phgfoundation.org/pages/work.htm
Standards Council of Canada (SCC)	www.scc.ca/en/
UNESCO	www.unesco.org
United Kingdom Accreditation Service (UKAS)	www.ukas.org
Wadsworth Center – NY Department of Health	http://www.wadsworth.org/labcert/clep/clep.html

Appendix B. Catalogue of Molecular Tests Relevant to Medicare Over-65-year Population

Appendix B includes the following:

1) Molecular test information available from the AMP test directory
2) Guide to molecular test tables
3) List of laboratories from which test information was collected
4) Table of abbreviations used in the tables
5) Laboratory-developed molecular test tables
6) Commercially available molecular test tables
7) FDA-approved molecular tests

Table 11. Molecular Test Information Available from the AMP Test Directory

Condition	Information
Infectious disease	• Type of infectious agent (e.g., bacterial, viral, fungal, or parasitic) • Infectious agent group (e.g., staphylococcus, enterovirus, etc.) • Infectious agent (e.g., methicillin resistance, echovirus, etc.) • Disorder/use of test (e.g., diagnosis, typing, etc.) • Assay type (e.g., qualitative, quantitative, etc.) • Availability (e.g., clinical, research, or both) • Laboratory name and location • Link to laboratory Web site and specific information about test
Solid Tumor	• Test category (e.g., amplification, mutation analysis, etc.) • Gene (e.g., *HER2/neu/ERBB2*) • Chromosome • Tumor type (e.g., breast cancer) • Assay type (e.g., qualitative, quantitative) • Availability (e.g., clinical, research, or both) • Laboratory name and location • Link to laboratory Web site and specific information about test
Hematopathology	• WHO category (e.g., acute myeloid leukemia, B and T cell neoplasms) • WHO abnormality (e.g., t(14;18)(q32;q21) or variant) • Gene (e.g., *BCL-2*) • Chromosome (e.g., t(14;18)(q32;q21)) • Test use (e.g., classification, diagnosis, prognosis) • Assay type (e.g., qualitative, quantitative) • Availability (e.g., clinical, research, or both) • Laboratory name and location • Link to laboratory Web site and specific information about test

WHO World Health Organization

Guide to Molecular Test Tables

As noted previously, the primary source for the molecular tests included in the following tables was the Web site of the AMP. Laboratories voluntarily submit information regarding tests they perform; consequently, not all laboratories or all molecular tests are represented. Overall, we created nine tables of which five include only laboratory-developed tests and four include tests performed using commercial kits or commercially available products (e.g., ASRs). Each table is specific to the following clinical conditions: infectious diseases, solid tumors, and hematopathology. For infectious diseases, separate tables were created for diseases caused by bacterial agents, viral agents, or fungal or parasitic agents. The exact organization of the tables varies depending on the clinical condition. All tables include information about the type of test method used (e.g., polymerase chain reaction [PCR], fluorescence in situ hybridization [FISH]), the primary use of the test (e.g., diagnosis, prognosis, monitoring, or treatment decisions), the assay type (e.g., qualitative or quantitative), and the laboratory identification number. In an effort to conserve space and aid the reader, we assigned identification numbers to the laboratories, instead of listing each laboratory that performed a test by name. The name and identification number of each laboratory for which we obtained testing information are presented in Table 12. Common abbreviations used to describe the testing methods are listed in Table 13.[6]

The tables are further organized by condition using the following categories:

Infectious Disease

The infectious disease tables are organized using the following seven column headings: infectious agent, test use, test method, assay type, source (only for tests performed using commercial kits or products), laboratory identification, and total number of laboratories that perform test.

Solid Tumors

The solid tumor tables are organized using the following seven column headings: gene, tumor type/subtype, test use, test method, assay type, laboratory identification, and total number of laboratories that perform tests.

Hematopathology

The hematopathology tables are organized using the following seven column headings: gene, gene specifics, disorder/test use, test method, assay type, laboratory, and number of laboratories. Each table is further organized by World Health Organization (WHO) categories. The following WHO categories are included: mastocytosis & myelodysplastic, acute myeloid leukemia's, B and T cell neoplasms, chronic myeloproliferative diseases, histiocytic and dendritic-cell neoplasms, and Hodgkin lymphoma.

[6] Some tests were excluded because they were not performed using a molecular method (e.g., immunohistology).

List of Laboratories

Table 12. Laboratory Name and Identification Number

Laboratory Name	Identification Number
Advocate Lutheran General Hospital	1
Albany Medical Center	2
Ambry Genetics Corp	3
Ameripath Center For Advanced Diagnostics	4
Armed Forces Institute of Pathology	5
ARUP Laboratories	6
Association for Molecular Pathology Corporate Laboratory	7
Athena Diagnostics Inc.	8
Barnes-Jewish Hospital	9
BayCare Laboratories	10
Baylor College of Medicine	11
Baylor University Medical Center	12
Berkshire Medical Center	13
Cenetron Diagnostics	14
Center for Human Genetics	15
Children's Hospital (Akron)	16
Children's Hospital Medical Center (Columbus)	17
Children's Hospital (Denver)	18
Children's Hospital (Philadelphia)	19
Christiana Care Health Services	20
Clinical Molecular Diagnostic Laboratory	21
Columbia Presbyterian Medical Center	22
Comprehensive Genetic Services	23
Cook Children's Medical Center	24
Correlagen Diagnostics Inc.	25
Creighton University Medical Center	26
Dana Farber Cancer Center	27
Dartmouth-Hitchcock Medical Center	28
Duke University Medical Center	29
Emory Medical Laboratory	30
Evanston Northwestern Healthcare	31
Florida Hospital	32
Focus Technologies	33

Laboratory Name	Identification Number
Fox Chase Cancer Center	34
GeneDx	35
Genomic Health, Inc.	36
Huntington Medical Research Institute	37
Investigen	38
Johns Hopkins Hospital	39
LabCorp	40
LDS Hospital / Intermountain Healthcare	41
Louisiana State University Health Sciences Center	42
Mayo Clinic -Cytogenetic Lab	43
Mayo Clinic-Molecular Genetics Lab	44
MD Anderson Cancer Center	45
Medical University of South Carolina	46
Molecular Pathology Laboratory Network, Inc	47
Myriad Genetic Laboratories	48
New York Presbyterian Hospital	49
North Shore Long Island Jewish Health System	50
Ohio State University	51
Oregon Health & Sciences University	52
Pathology Associates Medical Laboratories	53
PhenoPath Laboratories	54
Quest Diagnostics	55
RedPath Integrated Pathology, Inc.	56
Regional Medical Laboratory	57
Reliagene Technologies	58
Rhode Island Hospital	59
Roche Diagnostic	60
Rush Presbyterian St. Luke's Medical Center	61
Sacred Heart Medical Center	62
Saint Barnabus Medical Center	63
Saint Louis University School of Medicine	64
Specialty Laboratory	65
Southern Arizona VA Health Care System	66
Targeted Diagnostics & Therapeutics, Inc.	67
Targeted Molecular	68
Texas Children's Hospital	69

Laboratory Name	Identification Number
The Methodist Hospital	70
Thomas Jefferson University	71
UCLA Medical Center	72
UMDNJ / NJ Medical School	73
UNC Hospitals	74
University of Alabama Hospital	75
University of California Irvine Medical Center	76
University of Colorado Hospital	77
University of Iowa Hospital & Clinics	78
University of Nebraska Medical Center	79
University of Oklahoma Health Sciences Center	80
University of Pennsylvania Health System	81
University of Pittsburgh Medical Center	82
University of South Alabama	83
University of Texas Medical Branch	84
University of Utah School of Medicine	85
University of Washington Medical Center	86
University of Texas Southwestern Medical Center	87
Upstate Medical University	88
Vanderbilt University Medical Center	89
VCU Medical Center	90
Washington Hospital Center	91
William Beaumont Hospital	92
Yale New Haven Hospital	93
Yale University of Medicine	94
York Hospital	95
Total Number of Laboratories	**95**

Abbreviations Used in Molecular Test Tables

Table 13. Abbreviations for Testing Methods

Abbreviation	Description
ASPE	Allele Specific Primer
bDNA	Branched-chain DNA
BLA	Biological License Application
FISH	Fluorescence In Situ Hybridization
QC	Quality Control
Qual	Qualitative
Quant	Quantitative
HPA	Hybridization Protection
HYB	Hybridization
LCR	Ligase Chain Reaction
LIPA	Lipase A
LOH	Loss of Allele
NASBA	Nucleic Acid Sequence Based Amplification
PCR	Polymerase Chain Reaction
PCR-ASO blot	Polymerase Chain Reaction Allele-Specific Oligonucleotide
PCR-SSCP	Polymerase Chain Reaction-Single Strand Conformation Polymorphism
PFGE	Pulsed Field Gel Electrophoresis
Rep-PCR	Repetitive extragenic palindromic-PCR
RFLP	Restriction Fragment Length Polymorphism
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SDA	Strand Displacement Amplification
TC	Target Capture
TMA	Transcription Mediated Amplification

Laboratory-Developed Molecular Tests

Table 14. Laboratory-Developed Molecular Tests: Bacterial Infectious Diseases

Infectious Agent	Test Use	Method	Assay Type	Laboratory*	Number of Laboratories
Bacteria, identification	Diagnosis	PCR, sequencing	Genotype	69, 86	2
Bacteria, unspecified	Diagnosis (for bacteria identification)	PCR, sequencing	Genotype	69, 86, 55, 40, 65	5
Bacteria, unspecified	Diagnosis (for molecular typing)	PCR, sequencing, ribotyping, rep-PCR	Quant	20, 39	2
Bartonella henselae	Diagnosis	PCR, real-time PCR, HYB, sequencing	Qual, genotype	17, 33, 86, 89, 40, 55, 65	7
Bartonella quintana	Diagnosis	PCR, real-time PCR, sequencing	Qual, genotype	33, 86, 40, 65	4
Bartonella species	Diagnosis	PCR	Qual	39, 55	2
Bordetella pertussis	Diagnosis	PCR, real-time PCR, RFLP, HYB	Qual	2, 16, 17, 18, 24, 28, 87, 40, 55, 65	10
Bordetella pertussis	Diagnosis	PCR, gel	Qual	39	1
Bordetella pertussis and Bordetella parapertussis	Diagnosis	Real-time PCR	Qual	6, 33, 62, 73, 40, 55, 65	7
Borrelia burgdorferi	Diagnosis	PCR, HYB	Qual	6, 13, 33, 39, 40, 55, 65	7
Chlamydia pneumoniae	Diagnosis	PCR, real-time PCR, HYB	Qual	2, 17, 33, 39, 41, 77, 89, 40, 55, 65	10
Clostridium difficile	Diagnosis	PCR	Genotype	86, 40, 55, 65	4
Clostridium difficile	Diagnosis	Real-time PCR	Qual	33	1
Ehrlichia chaffeensis (HME) and anaplasma phagocytophilium (HE)	Diagnosis	PCR, real-time PCR	Qual	33, 39, 89, 40, 55, 65	6
Enterococcus, vancomycin resistance VRE vanA, vanB	Treatment decisions, prognosis	PCR, real-time PCR	Qual	17, 24, 39, 78, 40, 55, 65	7

Infectious Agent	Test Use	Method	Assay Type	Laboratory*	Number of Laboratories
Escherichia coli, EHEC E coli VTI/VTII Enterohemorrhagic E coli O157:H7	Diagnosis	Real-time PCR	Qual	69, 40, 55, 65	4
Helicobacter pylori	Diagnosis	PCR	Qual	69, 40, 55, 65, 71	5
Legionella pneumophila	Diagnosis	PCR, real-time PCR, HYB	Qual	33, 39, 41, 40, 55, 65	6
Legionella species	Diagnosis	PCR, HYB	Qual	2, 6, 40, 55, 65	5
Legionella species	Diagnosis	Real-time PCR	Qual	33	1
Methicillin resistance mecA	Treatment decisions, prognosis	PCR	Not specified	1, 40, 55, 65	4
Mycobacterium avium	Diagnosis	PCR, RFLP	Qual	10, 55, 65	3
Mycobacterium avium complex MAC	Diagnosis	PCR, RFLP	Qual	10, 65	2
Mycobacterium gordonae	Diagnosis	PCR, RFLP	Qual	10	1
Mycobacterium kansasii	Diagnosis	PCR, RFLP	Qual	10	1
Mycobacterium tuberculosis MTB	Diagnosis	PCR, real-time PCR, RFLP	Qual	10, 30, 31, 89, 40, 55, 65	7
Mycobacterium tuberculosis, drug resistance	Treatment decisions, prognosis	PCR, sequencing	Genotype	33, 40, 55, 65	4
Mycobacterium, rapidly growing	Diagnosis	PCR, sequencing	Qual, Genotype	6, 86, 65	3
Mycoplasma genitalium	Diagnosis	PCR, HYB	Qual	2	1
Mycoplasma hominus	Diagnosis	PCR, HYB	Qual	2	1
Mycoplasma pneumoniae	Diagnosis	PCR, real-time, HYB	Qual	2, 6, 16, 17, 33, 39, 77, 40, 55, 65	10

Infectious Agent	Test Use	Method	Assay Type	Laboratory*	Number of Laboratories
Mycoplasma species/culture confirmation	Diagnosis	PCR, sequencing	Qual	6, 39, 40, 55, 65	5
Rickettsia	Diagnosis	Real-time PCR	Qual	33	1
Shiga like toxins 1 & 2 SLT1 and SLT2 Stx1 and Stx2	Diagnosis	PCR	Qual	79, 40, 55, 65	4
Staphylococcus aureus, methicillin resistance MRSA mecA	Treatment decisions, prognosis	PCR	Qual	2, 6, 17, 22, 24, 31, 41, 58, 77, 40, 55, 65	12
Tropheryma whippelii	Diagnosis	Real-time PCR	Qual	33, 55, 65	3
Ureaplasma urealyticum mycoplasma hominis mycoplasma genitalium	Diagnosis	PCR, HYB	Qual	2, 40, 55, 65	4

* Refer to Table 12 for Identification Number of Laboratory.

Table 15. Laboratory Developed Molecular Tests: Viral Infectious Diseases

Infectious Agent	Test Use	Method	Assay Type	Laboratory*	Number
Acute viral hepatitis panel, HAV, HBC, HCV	Diagnosis	RT, PCR	Qual	38, 65	2
Adenovirus respiratory virus	Diagnosis	PCR, sequencing	Genotype	39, 65	2
Adenovirus respiratory virus	Diagnosis	PRC, real-time PCR, HYB	Quant, Qual	5, 33, 55, 39, 77, 79, 82	7
BK virus	Treatment decisions, prognosis	Real-time PCR	Quant	2, 30, 31, 33, 55, 39, 65, 77, 79, 82	10
C-C-chemokine receptor type 2, 64I variant CCR2-64I	Diagnosis, prognosis	PCR, NASBA	Qual	50	1
Cytomegalovirus (CMV)	Treatment decisions, prognosis	PCR, real-time PCR, HYB, RT, in situ	Qual, Quant	1, 2, 6, 12, 17, 33, 39, 40, 41, 52, 58, 59, 64, 65, 71, 74, 77, 79, 80, 82, 87, 89, 90, 91	24
Cytomegalovirus, foscarnet resistance CMV	Treatment decisions, prognosis	PCR, sequencing	Genotype	77	1
Cytomegalovirus, ganciclovir, foscarnet and cidofovir resistance CMV	Treatment decisions, prognosis	PCR, sequencing	Genotype	33, 39, 77	3
Dengue virus	Diagnosis	Real-time RT, PCR	Qual	33, 65	2
Enterovirus echovirus coxsackievirus	Diagnosis	Real-time RT, PCR, real-time PCR, sequencing, HYB	Qual	2, 5, 14, 16, 17, 24, 30, 31, 33, 39, 40, 41, 64, 65, 73, 77, 78, 79, 87, 89	20
Epstein-Barr virus (EBV)	Treatment decisions, prognosis	PCR, real-time PCR, HYB, in situ	Qual, Quant	6, 12, 14, 17, 30, 31, 33, 55, 39, 41, 49, 58, 59, 65, 71, 73, 74, 75, 77, 79, 80, 82, 87, 89, 90, 91, 93	27
Hepatitis A virus (HAV)	Diagnosis	RT, PCR, PFGE	Qual	38, 65	2
Hepatitis B virus (HBV)	Diagnosis, prognosis	PCR, real-time PCR, RFLP, sequencing, HYB	Qual, Quant	2, 33, 55, 38, 65, 71, 82	7

Infectious Agent	Test Use	Method	Assay Type	Laboratory*	Number
Hepatitis C virus (HCV)	Diagnosis	PCR, real-time PCR, HYB, RT	Qual, Quant	55, 38, 58, 65, 71, 87	6
Hepatitis C virus, genotyping HCV	Diagnosis, prognosis, treatment decisions	RT, PCR, sequencing	Genotype	39, 82	2
Hepatitis D virus (HDV)	Diagnosis	Real-time RT, PCR	Qual	33, 65	2
Hepatitis G virus (HGV)	Diagnosis	RT, PCR, HYB	Qual	33	1
Herpes simplex virus (1 & 2) HSV1; HSV2	Diagnosis, prognosis	PCR, real-time PCR, HYB, in situ	Qual, Quant	1, 2, 5, 6, 12, 14, 16, 17, 24, 28, 30, 31, 33, 39, 40, 41, 58, 59, 64, 65, 73, 74, 77, 78, 79, 82, 83, 87, 89, 90, 91, 93	32
Herpes virus panel CMV, HSV1, HSV2, EBV, VZV, HHV6	Diagnosis	PCR	Qual	79	1
HTLV I/II	Diagnosis	PCR, real-time PCR, HYB	Qual	33, 49, 50, 82	4
Human herpes virus 6 (HHV-6)	Diagnosis, prognosis	PCR, real-time PCR, HYB	Qual, Quant	12, 71, 33, 55, 58, 77, 87, 89	8
Human herpes virus 7 (HHV-7)	Diagnosis, prognosis	PCR, real-time PCR, HYB	Qual, Quant	12, 33, 58, 77, 89	5
Human herpes virus 8 (HHV-8) KSHV	Diagnosis, prognosis	RT, PCR, real-time PCR, HYB	Qual, Quant	5, 12, 33, 55, 49, 50, 58, 87, 89, 64, 71, 77, 79	13
Human immunodeficiency virus 1 (HIV-1)	Diagnosis	PCR, HYB, RT-PCR, sequencing	Qual, Quant	6, 14, 17, 30, 55, 58, 65	7
Human immunodeficiency virus 2 (HIV-2)	Diagnosis	PCR, HYB	Qual	33, 65	2
Human metapneumovirus	Diagnosis	Real-time RT-PCR	Qual	33, 65	2
Human papillomavirus (HPV)	Diagnosis, prognosis	PCR, HYB, in situ	Qual	6, 50, 58, 65, 80, 87	6
Influenza A & B respiratory virus	Diagnosis	Real-time RT-PCR, real-time PCR, HYB	Qual	5, 33, 65, 89	4
JC virus	Diagnosis, prognosis	PCR, real-time PCR, HYB	Qual, Quant	2, 33, 55, 39, 40, 59, 65, 77, 79, 82	10

Infectious Agent	Test Use	Method	Assay Type	Laboratory*	Number
Morbillivirus	Diagnosis	Real-time RT, PCR	Qual	5	1
Norwalk virus	Diagnosis	Real-time RT, PCR	Qual	33, 40	2
Parechovirus	Diagnosis	Real-time RT, PCR, HYB	Qual	33, 39	2
Parvovirus	Diagnosis	PCR, real-time PCR, HYB, in situ, southern blot	Qual	6, 16, 17, 31, 33, 39, 40, 75, 77, 79, 87, 89	12
Respiratory virus, panel	Diagnosis	RT, PCR, HYB	Qual	6	1
RSV, influ A, parainflu123 Influenza A Respiratory virus	Diagnosis	Real-time RT, PCR	Qual	33, 65, 77, 89	4
SARS coronavirus	Diagnosis	Real-time RT, PCR	Qual	33, 65, 74	3
Stromal cell-derived factor 1, mutation analysis SDF-1	Prognosis, treatment decisions	PCR	Qual	50	1
Varicella zoster virus (VZV)	Diagnosis	PCR, real-time PCR, real-time RT, gel, HYB	Qual, Quant	2, 6, 12, 17, 31, 33, 39, 41, 58, 77, 79, 82, 87, 89, 93	15
West Nile virus	Diagnosis	Real-time PCR	Qual	14, 33, 40, 65, 79	5

* Refer to Table 12 for Identification Number of Laboratory.

Table 16. Laboratory-Developed Molecular Tests: Parasitic or Fungal Infectious Diseases

Infectious Agent	Test Use	Method	Assay Type	Laboratory*	Number
Aspergillis, screening	Diagnosis	Real-time PCR	Qual	33	1
Babesia microti	Diagnosis	Real-time PCR	Qual	33, 55, 40, 65	4
Blastomyces dermatitidis	Diagnosis, prognosis	Probe hybridization	Qual	65	1
Coccidioides immitis	Diagnosis, prognosis	Probe hybridization	Qual	65	1
Encephalitozoon species microsporidia	Diagnosis	Real-time PCR	Qual	66	1
Histoplasma capsulatum	Diagnosis, prognosis	Real-time PCR	Qual	33, 65	2
Mold, identification		PCR, sequencing	Genotype	86	1
Pneumocystis carinii	Diagnosis, prognosis	PCR	Qual	58, 64, 71	3
Toxoplasma gondii	Diagnosis	PCR, HYB	Qual	5, 6, 31, 33, 40, 58	6
Trichomonas vaginalis	Diagnosis	Real-time PCR	Qual	31, 55, 40, 65	4
Yeast, identification	Diagnosis	PCR, sequencing	Genotype	60, 86	2

* Refer to Table 12 for Identification Number of Laboratory.

Table 17. Laboratory-Developed Molecular Tests: Solid Tumors

Gene	Tumor Type/Subtype	Test Use	Test Method	Assay Type	Laboratory*	Number of Laboratories
APC	Colorectal cancer, FAP	Diagnosis	PCR-sequencing, Other	Qual	44, 63, 85	3
APC-specific mutation I1307K	Colorectal cancer, Ashkenzai Jewish	Diagnosis	Other	Qual	39, 63	2
BRCA1/BRCA2	Breast cancer	Diagnosis	PCR-sequencing, PCR-allele specific, Other	Qual	34, 74, 85	3
BRAF	Colon cancer	Diagnosis	Real-time PCR	Qual	6, 45, 82	3
C-kit	Gastrointestinal stromal tumor	Diagnosis	PCR-sequencing, real-time PCR, other	Qual	5, 6, 34, 55, 40, 42, 52	7
Cyclin D1/CCND1/PRAD1	Head & neck cancer, squamous cell cancer	Diagnosis, prognosis	Real-time PCR	Quant	5, 45	2
DCC	Colorectal cancer	Prognosis	Other	Qual	27, 63	2
DCC/SMAD4	Colorectal cancer	Diagnosis	PCR capillary gel electrophoresis	Qual	82	1
DPC4	Colorectal cancer	Prognosis	Other	Qual	63	1
EGFR	Lung cancer, non-small cell lung, glioblastoma, colon cancer	Diagnosis	PCR-sequencing, FISH	Qual	34, 55, 40, 43, 65, 88	6
EWS	Ewing's sarcoma, clear cell sarcoma	Diagnosis	FISH	Qual	94	1
EWS-ATF1	Sarcomas, clear cell sarcoma	Diagnosis	PCR-sequencing, PCR-ASO blot	Qual	79, 81	2
EWS-EIAF	Sarcomas, Ewing's sarcoma	Diagnosis	PCR-sequencing	Qual	9	1

Gene	Tumor Type/Subtype	Test Use	Test Method	Assay Type	Laboratory*	Number of Laboratories
EWS-ERG	Sarcomas, Ewing's sarcoma	Diagnosis, Prognosis	Real-time PCR, PCR-sequencing, PCR-allele specific, PCR-ASO blot, Other	Qual, Quant	6, 9, 69, 18, 79, 81, 88	7
EWS-ETV1	Sarcomas, Ewing's sarcoma	Diagnosis	PCR-sequencing	Qual	9	1
EWS-FEV	Sarcomas, Ewing's sarcoma	Diagnosis	PCR-sequencing	Qual	9	1
EWSR1	Sarcomas, Ewing's/PNET, clear cell sarcoma	Diagnosis	FISH	Qual	88	1
EWS-WT1	Sarcomas, desmoplastic small round cell tumor	Diagnosis	Other, PCR-sequencing, PCR-allele specific, PCR-ASO blot	Qual	5, 9, 69, 81	4
FKHR	Sarcomas, alveolar rhabdomyosarcoma	Diagnosis	FISH	Qual	88	1
FUS-CHOP	Sarcomas, myxoid liposarcoma	Diagnosis	PCR-allele specific	Quant	69	1
GCC mRNA	Colorectal cancer, metastatic disease	Prognosis, monitoring, treatment decisions	Other	Quant	67	1
HER2/neu/ERBB2	Breast cancer	Diagnosis, prognosis	Real-time PCR, FISH	Qual, Quant	6, 55, 40, 65, 74, 74, 88	7
HRAS, codon 61	Thyroid cancer	Diagnosis	Real-time PCR	Qual	82	1
Human androgen receptor (*HUMARA*)	All tumors	Diagnosis	PCR-restriction digest	Qual	94	1
KIT	Sarcomas, GIST	Diagnosis	Real-time PCR	Quant	5, 45	2
KRAS, codon 12/13	Colorectal cancer, thyroid cancer, lung cancer, pancreatic cancer	Diagnosis	PCR-sequencing	Qual	82	1

Gene	Tumor Type/Subtype	Test Use	Test Method	Assay Type	Laboratory*	Number of Laboratories
KRAS/Ki-Ras	Lung cancer, non-small cell, endometrial cancer, pancreatic cancer, colorectal cancer	Diagnosis	PCR-sequencing, PCR-SSCP	Qual	27, 45, 94	3
KRAS2/Ki-Ras	Colorectal cancer, pancreatic cancer	Diagnosis	PCR-SSCP	Qual	63, 94, 45, 27	4
MGMT	Glioblastoma	Diagnosis	Real-time PCR	Quant	82	1
Mismatch repair genes	Endometrial cancer, colorectal cancer, HNPCC/Lynch syndrome	Diagnosis	Other, PCR-allele specific, PCR-sequencing	Qual	5, 27, 34, 39, 40, 42, 61, 94, 44, 72, 82	11
MLH1	Colorectal cancer, HNPCC/Lynch syndrome, endometrial cancer	Diagnosis	Real-time PCR, other	Quant, Qual	27, 55, 44, 45, 65, 11, 26, 51, 64, 72, 6, 82, 94	13
MSH2	Ovarian cancer, colorectal cancer, HNPCC/Lynch syndrome, endometrial cancer	Diagnosis	Southern blot, other	Qual	55, 40, 44, 65, 11, 26, 51, 64, 72, 6, 82, 94	12
MSH6/GTBP	Colorectal cancer, HNPCC/Lynch syndrome, endometrial cancer	Monitoring	Other	Qual	55, 40, 44, 65, 11, 26, 51, 64, 72, 6, 82, 94	12
NBL1	Sarcomas, GIST	Diagnosis	Real-time PCR	Quant	5	1
N-myc/MYCN	Neuroblastoma	Diagnosis, Prognosis	FISH, Southern blot, Other	Qual, Quant	44, 74, 88	3
NRAS, codon 61	Thyroid cancer	Diagnosis	Real-time PCR	Qual	82	1
p15, p16/CDKN2A/MTS1	Glioblastoma, astrocytoma	Diagnosis	FISH	Quant	43, 26	2
p53/TP53	Astrocytoma	Prognosis	Other	Qual	55, 40, 65, 89, 4, 27	6

Gene	Tumor Type/Subtype	Test Use	Test Method	Assay Type	Laboratory*	Number of Laboratories
PAX3-FKHR	Sarcomas, alveolar rhabdomyosarcoma	Diagnosis	Real-time PCR, PCR-sequencing, PCR-allele specific, PCR-restriction digest, PCR-ASO blot	Qual, Quant	5, 6, 9, 18, 69, 79, 81	7
PAX7-FKHR	Sarcomas, alveolar rhabdomyosarcoma	Diagnosis	Real-time PCR, PCR-sequencing, PCR-allele specific, PCR-restriction digest, PCR-ASO blot	Qual, Quant	5, 6, 9, 69, 79, 81	6
PDGFB-COL1A1	Sarcomas, dermatofibrosarcoma protuberans	Diagnosis	PCR-allele specific	Quant	69	1
PDGFRa	Gastrointestinal stromal tumor	Diagnosis	Other	Qual	6	1
PMS2	Colorectal cancer, HNPCC/Lynch syndrome, endometrial cancer	Diagnosis	Other	Quant	44, 11, 26, 51, 64, 72, 94	7
PRSS1	Pancreatic cancer, hereditary pancreatitis	Diagnosis	PCR-sequencing	Qual	44	1
PTEN	Astrocytoma	Diagnosis	FISH	Quant	43, 51	2
RET proto-oncogene	MEN 2A, MEN 2B	Diagnosis	PCR-sequencing, PCR-restriction digest	Qual	5, 9, 55, 40, 44, 65, 85, 51, 19, 82	10
RET/PTC1 and RET/PTC3	Thyroid cancer, thyroid papillary carcinoma	Diagnosis	Real-time PCR	Qual	82	1
SYT	Sarcomas, synovial sarcoma	Diagnosis	FISH	Qual	88	1
SYT-SSX1	Sarcomas, synovial sarcoma	Diagnosis	Real-time PCR, PCR-sequencing, PCR-allele specific, PCR-ASO blot, FISH	Qual, Quant	5, 6, 9, 69, 79, 81, 94, 54	8

Gene	Tumor Type/Subtype	Test Use	Test Method	Assay Type	Laboratory*	Number of Laboratories
SYT-SSX2	Sarcomas, synovial sarcoma	Diagnosis	Real-time PCR, PCR-sequencing, PCR-allele specific, PCR-ASO blot	Qual	5, 6, 9, 69, 79, 81, 94, 54	8
Unknown	Oligodendroglioma, glioblastoma, astrocytoma	Diagnosis	Other, FISH	Qual, Quant	29, 39, 43, 88, 89, 82, 87, 94	8
VHL	Various	Diagnosis	Southern blot, PCR-sequencing	Qual	5, 19	2
DBC1	Bladder cancer	Prognosis	Not specified	Not specified	42	1
L-myc	Lung cancer, small cell carcinoma, breast cancer	Diagnosis, prognosis	Not specified	Not specified	42	1
p15/CDKN2B	Pancreatic cancer	Diagnosis	Not specified	Not specified	42	1

* Refer to Table 12 for Identification Number of Laboratory.

Table 18. Laboratory-Developed Molecular Tests: Hematopathology

WHO Category	Gene	Gene Specifics	Test Use	Test Method	Assay Type	Laboratory*	Number of Laboratories
Mastocytosis & Myelodysplastic	*BCR-ABL*	*BCR-ABL micro/mu-bcr/P230, BCR-ABL minor/m-bcr/P190, BCR-ABL major/m-bcr/P210,* unspecified	Diagnosis, prognosis, monitoring	RT-PCR, real-time PCR, FISH	Qual, Quant	81, 9, 5, 46, 61, 40, 55, 65, 39, 18, 74, 86, 90	13
Mastocytosis & Myelodysplastic	*KIT (SCFR)*	Unspecified	Diagnosis, Prognosis	PCR for specific mutation detection	Qual	42, 44	2
Mastocytosis & Myelodysplastic	*MLL(HRX)*	Unspecified	Diagnosis, prognosis	FISH	Quant	46, 40, 55, 65, 42	5
Mastocytosis & Myelodysplastic	*Not specified*	Unspecified	Diagnosis, prognosis	Conventional cytogenetics/karyotype, FISH	Quant, Qual	42, 46	2
Mastocytosis & Myelodysplastic	*PDGFRB-TEL (ETV6)*	Unspecified	Diagnosis, prognosis	Conventional cytogenetics/karyotype	Quant	44	1
Acute Myeloid Leukemias	*AF9-MLL(HRX)*	Unspecified	Diagnosis, prognosis	Conventional cytogenetics/karyotype, FISH	Qual, Quant	42, 46, 40, 55, 65	5
Acute Myeloid Leukemias	*AML 1(RUN1)(CBFa)/ETO*	Unspecified	Diagnosis, prognosis	FISH	Quant	46, 18	2
Acute Myeloid Leukemias	*BCR-ABL*	Unspecified, *BCR-ABL*, *BCR-ABL major/M-bcr/P210, BCR-ABL minor/M-bcr/P190*	Diagnosis, prognosis, monitoring	RT-PCR, real-time PCR, FISH, PCR	Qual, Quant	9, 29, 46, 47, 61, 79, 5, 81, 90, 40, 55, 65, 6, 39, 74, 18, 86	17
Acute Myeloid Leukemias	*CBFb/MYH11*	Unspecified	Diagnosis, prognosis	FISH, RT-PCR	Quant, Qual	46, 55, 18	3
Acute Myeloid Leukemias	*DEK-CAN*	Unspecified	Prognosis	Conventional cytogenetics/karyotype	Qual, Quant	42, 43	2
Acute Myeloid Leukemias	*ETO (CBFA2T1)-AML1 (RUNX1orCBFA2)*	Unspecified	Diagnosis, prognosis	FISH	Quant	46, 18	2

WHO Category	Gene	Gene Specifics	Test Use	Test Method	Assay Type	Laboratory*	Number of Laboratories
Acute Myeloid Leukemias	Ig Heavy/Light	Ig heavy chain/IgH/14q32, unspecified	Diagnosis, prognosis, monitoring	PCR, FISH	Qual, Quant	47, 46, 5, 80, 81, 40, 6, 39, 63, 74, 18, 78, 86	13
Acute Myeloid Leukemias	*MLL(HRX)*	Unspecified	Diagnosis, prognosis	Conventional cytogenetics/karyotype, FISH	Qual, Quant	42, 46, 47, 40, 55, 65	6
Acute Myeloid Leukemias	*MLL(HRX)-ELL*	Unspecified	Prognosis	Conventional cytogenetics/karyotype, FISH	Qual, Quant	42, 46, 40, 55, 65	5
Acute Myeloid Leukemias	*MLL(HRX)-ENL*	Unspecified	Diagnosis, prognosis	Conventional cytogenetics/karyotype, FISH	Qual, Quant	42, 46, 40, 55, 65	5
Acute Myeloid Leukemias	*MOV-CBP(CREBBP)*	Unspecified	Diagnosis, prognosis	Conventional cytogenetics/karyotype, FISH	Qual, Quant	42, 46	2
Acute Myeloid Leukemias	Not specified	Unspecified	Diagnosis, prognosis	Conventional cytogenetics/karyotype, FISH	Qual, Quant	42, 46, 47	3
Acute Myeloid Leukemias	*NPM-RARa*	Unspecified	Diagnosis, prognosis	Conventional cytogenetics/karyotype, FISH	Qual, Quant	42, 46	2
Acute Myeloid Leukemias	*NUMA-RARa*	Unspecified	Diagnosis, prognosis	Conventional cytogenetics/karyotype, FISH	Qual, Quant	42, 46	2
Acute Myeloid Leukemias	*PLZF-RARa*	Unspecified	Prognosis	Conventional cytogenetics/karyotype, FISH	Qual, Quant	42, 46	2
Acute Myeloid Leukemias	*PML-RARa*	Unspecified	Diagnosis, prognosis, monitoring	RT-PCR, real-time PCR, FISH	Qual, Quant	9, 30, 46, 47, 81, 40, 55, 6, 44, 18	10

WHO Category	Gene	Gene Specifics	Test Use	Test Method	Assay Type	Laboratory*	Number of Laboratories
Acute Myeloid Leukemias	*STAT5B-RARa*	Unspecified	Diagnosis, prognosis	Conventional cytogenetics/karyotype, FISH	Qual, Quant	42, 46	2
Acute Myeloid Leukemias	*TCR* genes	*TCR* gamma/7p15, unspecified	Prognosis	PCR	Qual	47, 5, 81, 40, 55, 65, 6, 39, 44, 63, 74, 18, 78	13
Acute Myeloid Leukemias	*TEL(ETV6)*	Unspecified	Diagnosis, prognosis	FISH	Quant	46, 65, 55	3
B and T Cell Neoplasms	*ATM*	Unspecified	Diagnosis, prognosis	FISH	Quant	46	1
B and T Cell Neoplasms	*BCL-1*	Unspecified, *BCL-1 MTC* region, *BCL-1* unspecified breaking point	Diagnosis, prognosis	FISH, PCR	Quant, Qual	46, 40, 55, 65, 6, 86	6
B and T Cell Neoplasms	*BCL-1 (PRAD1 or CCND1 or cyclin D1)*	*BCL-1 MTC* and outside of *MTC, BCL-1* unspecified breakpoint, unspecified, *BCL-1 MTC* region	Diagnosis, prognosis	RT-PCR, real-time PCR, PCR	Quant, Qual	5, 46, 94, 40, 55, 65, 6, 86	8
B and T Cell Neoplasms	*BCL-1-Igh*	*BCL-1 MTC* and outside of *MTC, BCL-1* unspecified breakpoint, *BCL-1 MTC* region, unspecified	Diagnosis, prognosis, monitoring	RT-PCR, real-time PCR, PCR	Quant, Qual	5, 46, 40, 55, 65, 6, 86	7
B and T Cell Neoplasms	*BCL-2*	*BCL-2 MBR, BCL-2 mcr,* unspecified, *BCL-2* unspecified breaking point	Diagnosis, prognosis, monitoring	RT-PCR, PCR, real-time PCR, FISH	Qual, Quant	5, 9, 30, 46, 47, 61, 81, 89, 90, 94, 40, 55, 65, 6, 72, 78, 86	17
B and T Cell Neoplasms	*BCL-3*	*BCL-2 MBR, BCL-2 mcr*	Prognosis	Real-time PCR	Qual	30	1
B and T Cell Neoplasms	*BCL-6(LAZ3)*	Unspecified	Diagnosis, prognosis	FISH	Quant	46, 65	2

WHO Category	Gene	Gene Specifics	Test Use	Test Method	Assay Type	Laboratory*	Number of Laboratories
B and T Cell Neoplasms	*BCR-ABL*	Unspecified, *BCR-ABL* major/M-bcr/P210, *BCR-ABL* minor/m-bcr/P190, *BCR-ABL* breaking point not specified	Diagnosis, prognosis, monitoring	RT-PCR, FISH, real-time PCR	Qual, Quant	5, 9, 46, 81, 90, 40, 39, 6, 74, 80, 86	11
B and T Cell Neoplasms	*BIRC3(API2)-MALT1(MLT)*	Unspecified	Diagnosis, prognosis	FISH, PCR, PCR for specific mutation detection	Quant, Qual	46, 40, 55, 65, 6, 43	6
B and T Cell Neoplasms	*EBV*	Unspecified	Diagnosis, prognosis, monitoring	PCR, RT-PCR, FISH, real-time PCR, Southern	Qual, Quant	5, 46, 94, 40, 55, 65, 43, 74, 18, 78	10
B and T Cell Neoplasms	*HHV-8/KSHV* viral	Unspecified	Diagnosis, prognosis, monitoring	RT-PCR, PCR	Qual	5, 94, 65	3
B and T Cell Neoplasms	Ig Heavy/Light	Ig heavy chain/IgH/14q32, unspecified, Ig light chain/IgK/2p12, Ig light chain/IgL/22q11.2, Ig(unspecified gene)	Diagnosis, prognosis, monitoring	PCR, FISH, RT-PCR, Southern	Qual, Quant	5, 9, 46, 47, 61, 80, 81, 89, 94, 40, 39, 6, 74, 18, 78, 86, 47	17
B and T Cell Neoplasms	*MLL(HRX)*	Unspecified	Diagnosis, prognosis	FISH	Quant	46, 40, 55, 65	4
B and T Cell Neoplasms	*MYC*	Unspecified	Diagnosis, prognosis	FISH	Quant	46, 40, 55, 65	4
B and T Cell Neoplasms	*MYC-IgH* and variants	Unspecified	Diagnosis, prognosis	FISH	Qual, Quant	94, 46, 40, 55, 65	5
B and T Cell Neoplasms	not specified	Unspecified	Diagnosis, prognosis	FISH, PCR	Quant, Qual	46, 81, 89	3
B and T Cell Neoplasms	*NPM1-ALK* or other *ALK*	Unspecified	Diagnosis, prognosis, monitoring	RT-PCR, real-time PCR	Qual, Quant	5, 46, 40, 55, 65, 18	6
B and T Cell Neoplasms	*NPM-ALK*	Unspecified	Diagnosis, Prognosis, monitoring	PCR, FISH, real-time PCR, RT-PCR	Qual, Quant	5, 46, 40, 55, 65, 18	6

WHO Category	Gene	Gene Specifics	Test Use	Test Method	Assay Type	Laboratory*	Number of Laboratories
B and T Cell Neoplasms	*P53*	Unspecified	Diagnosis, prognosis	FISH	Quant	46, 40, 55, 65	4
B and T Cell Neoplasms	*PBX-E2A*	Unspecified	Diagnosis, prognosis	Conventional cytogenetics/karyotype, FISH, RT-PCR, real-time PCR	Qual, Quant	42, 46, 6, 43, 18	5
B and T Cell Neoplasms	*TCR* genes	Unspecified, *TCR* gamma/7p15	Diagnosis, prognosis, monitoring	PCR	Qual, Quant	5, 47, 61, 81, 89, 94, 40, 55, 65, 39, 6, 43, 74, 18, 78, 86	16
B and T Cell Neoplasms	*TEL(ETV6)/AML1*	Unspecified	Diagnosis, prognosis	Real-time PCR, RT-PCR	Qual	6, 18	2
Chronic Myeloproliferative Diseases	*BCR-ABL*	*BCR-ABL* major/M-bcr/P210, unspecified, *BCR-ABL* minor/m-bcr/P190, *BCL-ABL, BCR-ABL* micro/mu-bcr/P230, *BCR-ABL* breaking point not specified	Diagnosis, prognosis, monitoring	RT-PCR, real-time PCR	Qual, Quant	5, 9, 29, 52, 61, 80, 81, 90, 40, 55, 65, 39, 6, 42, 72, 74, 18, 86	18
Chronic Myeloproliferative Diseases	Ig Heavy/Light	Ig heavy chain/IgH/14q32, unspecified	Diagnosis, prognosis	PCR	Qual	80, 40	2
Chronic Myeloproliferative Diseases	*JAK2*	Unspecified	Diagnosis, prognosis	PCR	Qual	52, 55, 65	3
Chronic Myeloproliferative Diseases	*PDGFRB-TEL(ETV6)*	Unspecified	Prognosis	Conventional cytogenetics/karyotype	Qual, Quant	42, 43	2
Histiocytic and Dendritic-Cell Neoplasms	*HUMARA*	Unspecified	Diagnosis, prognosis, monitoring	PCR	Qual	89	1

WHO Category	Gene	Gene Specifics	Test Use	Test Method	Assay Type	Laboratory*	Number of Laboratories
Histiocytic and Dendritic-Cell Neoplasms	Ig Heavy/Light	Ig heavy chain/IgH/14q32, unspecified	Diagnosis, prognosis	PCR, RT-PCR , FISH, Southern	Qual	5, 46, 81, 40, 39, 6, 74, 18, 78, 86	10
Histiocytic and Dendritic-Cell Neoplasms	*TCR* genes	Unspecified	Diagnosis, prognosis	PCR, Southern	Qual, Quant	5, 81, 89, 40, 55, 65, 39, 6, 74, 18, 78, 86	12
Hodgkin Lymphoma	Ig Heavy/Light	Ig heavy chain/IgH/14q32, unspecified	Diagnosis, prognosis	PCR, FISH, PCR and mutation scanning	Qual, Quant	5, 46, 81, 40, 39, 6, 74, 18, 78, 80, 86, 90, 30	13

* Refer to Table 12 for Identification Number of Laboratory.

Molecular Tests Offered by Laboratories Using Commercial ASRs or Kits

Table 19. Tests Using Commercial Kits: Bacterial Infectious Diseases

Infectious Agent	Test Use	Method	Assay Type	Source of Kits	Laboratory*	Number
Bacteria, identification	Diagnosis	PCR, LiPA	Quant	Chemicon	7	1
Bacteria, molecular typing	Prognosis, treatment decisions	rep-PCR	Genotype	Bacterial Barcodes	69	1
Bacteria, molecular typing	Prognosis, treatment decisions	Probe hybridization	Semi-quant	Bayer (Innogenetics), 5' nc (UTR) region genotyping	7	1
Bacteria, molecular typing	Prognosis, treatment decisions	PFGE	Qual	Bio-Rad	2	1
Bacteria, molecular typing	Prognosis, treatment decisions	PFGE	Quant	Bio-Rad	20	1
Bacteria, molecular typing	Prognosis, treatment decisions	PFGE	Genotype	Epidemiologic Investigations	6, 22, 29, 31, 39, 77, 86, 93	8
Bacteria, molecular typing	Diagnosis	Rep-PCR	Genotype	bioMerieux	66	1
Bartonella quintana	Diagnosis	PCR	Quant	Epidemiologic Investigations	7	1
Bordetella pertussis	Diagnosis	Real-time PCR	Qual	Cepheid	79	1
Bordetella pertussis	Diagnosis	Real-time PCR	Qual	Pordessa	10	1
Chlamydia pneumonia/psittaci, legionella pneumophila and mycoplasma pneumoniae	Diagnosis	LCR	Quant	Biosource	7	1
Chlamydia trachomatis (CT)	Diagnosis	LCR	Qual	Abbott	6, 13, 53, 64, 74, 77, 83	7
Chlamydia trachomatis (CT)	Diagnosis	SDA	Qual	Becton-Dickinson	1, 2, 10, 16, 22, 30, 50, 75, 79	9

Infectious Agent	Test Use	Method	Assay Type	Source of Kits	Laboratory*	Number
Chlamydia trachomatis (CT)	Diagnosis	Direct probe	Qual	Genprobe	6, 50, 53, 58, 86, 93	6
Chlamydia trachomatis (CT)	Diagnosis	PCR, HYB	Qual	Roche	4, 18, 29, 31, 39, 41, 46, 66, 73, 74, 76, 77, 78, 91, 92	15
Escherichia coli, EHEC E coli VTI/VTII enterohemorrhagic E coli O157:H7	Diagnosis	Hybrid capture	Qual	Commercial	7	1
Methicillin resistance mecA	Treatment decisions	PCR, PFGE	Not specified	Bio-Rad	22	1
Mycobacterium avium	Diagnosis	PFGE	Quant	Bio-Rad	20	1
Mycobacterium avium complex MAC	Diagnosis	Direct probe	Qual	Genprobe	6, 29, 39, 50, 62, 66, 76, 77, 86, 73, 75, 93, 73	13
Mycobacterium avium complex MAC	Diagnosis	SDA	Qual	Becton Dickinson	75	1
Mycobacterium gordonae	Diagnosis	PFGE	Quant	Bio-Rad	20	1
Mycobacterium gordonae	Diagnosis	Direct probe	Qual	Genprobe	29, 39, 50, 62, 75, 76, 77, 86, 93	9
Mycobacterium kansasii	Diagnosis	Direct probe	Qual	Genprobe	29, 39, 50, 76	4
Mycobacterium tuberculosis MTB	Diagnosis	Direct probe, TMA	Qual	Genprobe	6, 22, 29, 39, 50, 62, 73, 76, 92, 93	10
Mycobacterium tuberculosis MTB	Diagnosis	PCR, HYB	Qual	Roche	33, 66, 86, 87	4
Mycobacterium tuberculosis, culture confirmation	Diagnosis	PCR	Qual	Genprobe	73	1
Mycobacterium tuberculosis, culture confirmation	Diagnosis	Direct Probe	Qual	Genprobe	1, 6, 29, 31, 39, 50, 66, 79, 86, 94, 73, 75, 76, 77, 93	15
Mycobacterium tuberculosis, molecular typing MTB	Diagnosis	RFLP	Genotype	Epidemiologic Investigations	39	1

Infectious Agent	Test Use	Method	Assay Type	Source of Kits	Laboratory*	Number
Mycobacterium, rapidly growing	Diagnosis	PFGE	Quant	Bio-Rad	20	1
Neisseria gonorrhoeae	Diagnosis	PCR, LCR	Qual	Abbott	6, 13, 53, 64, 77, 83, 86	7
Neisseria gonorrhoeae	Diagnosis	SDA	Qual	Becton-Dickinson	1, 2, 10, 16, 22, 30, 50 75, 79	9
Neisseria gonorrhoeae	Diagnosis	Direct probe	Qual	Genprobe	6, 50, 53, 58, 94, 93	6
Neisseria gonorrhoeae	Diagnosis	PCR, HYB	Qual	Roche	4, 18, 29, 31, 39, 41, 46, 66, 73, 74, 76, 91, 92	13
Staphylococcus aureus, methicillin resistance MRSA mecA	Treatment decisions	Real-time PCR	Qual	Infection Diagnostic	10	1
Staphylococcus aureus, methicillin resistance MRSA mecA	Diagnosis	PCR	Qual	Other	50	1
Staphylococcus aureus, methicillin resistance MRSA mecA	Treatment decisions	Real-time PCR	Qual	Roche	39, 69	2

* Refer to Table 12 for Identification Number of Laboratory.

Table 20. Tests Using Commercial Kits: Viral Infectious Diseases

Infectious Agent	Test Use	Method	Assay Type	Source of Kits	Laboratory*	Number
BK virus	Prognosis	Real-time PCR	Qual, Quant	Nanogen	32	1
Cytomegalovirus CMV	Prognosis	Real-time PCR	Quant	Abbott, In house	24	1
Cytomegalovirus CMV	Prognosis	PCR	Qual	Argene Biosoft	50	1
Cytomegalovirus CMV	Prognosis	NASBA	Qual	bioMerieux	50	1
Cytomegalovirus CMV	Prognosis	HYB	Semi-quant	Digene	16, 29, 53, 77	4
Cytomegalovirus CMV	Prognosis	Real-time RT, PCR	Quant	Nanogen	32	1
Cytomegalovirus CMV	Prognosis	PCR, real-time PCR, HYB	Qual, Quant	Roche	6, 14, 22, 30, 39, 66, 70, 73, 91	9
Enterovirus Echovirus Coxsackievirus	Diagnosis	NASBA	Qual	bioMerieux	50, 92	2
Enterovirus Echovirus Coxsackievirus	Diagnosis	NASBA	Qual	Organon-Teknika	93	1
Enterovirus Echovirus Coxsackievirus	Diagnosis	RT, PCR, HYB	Qual	Synthetic Genetics	1, 7, 18	3
Epstein-Barr virus EBV	Prognosis	Real-time PCR	Qual	Abbott, In house	24	1
Epstein-Barr virus EBV	Prognosis	PCR, HYB	Qual, Quant	Argene Biosoft	50	1
Epstein-Barr virus EBV	Prognosis	Real-time PCR	Qual	Nanogen	32	1
Epstein-Barr virus EBV	Prognosis	Real-time PCR	Quant	Roche	70	1
Hepatitis B virus HBV	Diagnosis, prognosis	HYB	Quant	Digene	73, 81	2
Hepatitis B virus HBV	Diagnosis, prognosis	PCR, real-time PCR, HYB	Quant	Roche	6, 14, 33, 39, 50, 66, 70, 76, 90, 92	10
Hepatitis B virus, drug resistance	Diagnosis, prognosis	PCR, LiPA	Genotype	Bayer	14	1

Infectious Agent	Test Use	Method	Assay Type	Source of Kits	Laboratory*	Number
Hepatitis B Virus, drug resistance	Diagnosis, prognosis, treatment decisions	PCR, sequencing	Genotype	Visible Genetics	14	1
Hepatitis C virus (HCV)	Diagnosis	PCR, real time, PCR, RT, real-time RT	Genotype, Qual, Quant	Abbott	78. 79, 84	3
Hepatitis C virus (HCV)	Diagnosis	bDNA, RT, PCR, HYB, TMA, LiPA	Qual, Quant	Bayer	6, 12, 32, 58, 73, 82, 92, 95	8
Hepatitis C virus (HCV)	Diagnosis	bDNA	Quant	Chiron	22, 53	2
Hepatitis C virus (HCV)	Diagnosis	RT, real-time RT, PCR, real-time PCR, HYB	Qual, Quant	Roche	1, 5, 6, 14, 29, 30, 31, 32, 33, 39, 41, 46, 50, 52, 62, 64, 66, 70, 73, 75, 76, 77, 81, 82, 83, 90, 91, 92, 93	29
Hepatitis C virus (HCV)	Diagnosis, prognosis, treatment decisions	Real-time PCR	Genotype	Third Wave	10	1
Hepatitis C virus, genotyping HCV	Diagnosis, prognosis, treatment decisions	PCR, RT, LiPA, RFLP, sequencing	Genotype	Bayer (Innogenetics), 5' nc (UTR) region genotyping	1, 2, 6, 14, 30, 33, 46, 52, 62, 73, 74, 75, 77, 79, 92, 93	16
Hepatitis C virus, genotyping HCV	Diagnosis, prognosis, treatment decisions	PCR, HYB	Genotype	Third Wave	90	1
Hepatitis C virus, genotyping HCV	Diagnosis, prognosis, treatment decisions	RT, PCR, sequencing	Genotype	Visible Genetics	50	1
Hepatitis C virus, ultrasensitive HCV	Diagnosis, prognosis, treatment decisions	Real-time RT, PCR	Quant	Abbott	10	1
Hepatitis C virus, ultrasensitive HCV	Diagnosis, prognosis	RT, PCR, real-time PCR	Qual, Quant	Roche	14, 33, 39, 78, 84	5
Herpes Simplex Virus (1 & 2) HSV1; HSV2	Diagnosis	PCR, HYB	Qual	Argene Biosoft	50	1
Herpes Simplex Virus (1 & 2) HSV1; HSV2	Diagnosis	Real-time PCR	Qual	Roche	18, 70, 87	3
Human Herpes Virus 6 HHV-6	Diagnosis	PCR, HYB	Qual	Argene Biosoft	50	1

Infectious Agent	Test Use	Method	Assay Type	Source of Kits	Laboratory*	Number
Human Immunodeficiency Virus 1 HIV-1	Diagnosis, prognosis	RT, PCR, real-time PCR, HYB, sequencing, bDNA	Qual, Quant	Roche	1, 6, 10, 14, 22, 29, 30, 31, 32, 33, 39, 50, 52, 58, 66, 70, 73, 74, 75, 76, 77, 78, 79, 87, 89, 90, 91, 92, 93	29
Human Immunodeficiency Virus 1 HIV-1	Diagnosis, prognosis	bDNA	Quant	Bayer	6, 32, 81, 84	4
Human Immunodeficiency Virus 1 HIV-1	Diagnosis, prognosis	NASBA	Quant	bioMerieux	2	1
Human Immunodeficiency Virus 1 HIV-1	Diagnosis, prognosis	bDNA	Quant	Chiron	53	1
Human Immunodeficiency Virus 1 HIV-1	Diagnosis, prognosis	NASBA	Quant	Organon-Teknika	50, 83	2
Human Immunodeficiency Virus 1 HIV-1, genotyping	Diagnosis, prognosis	PCR, sequencing	Genotype	Visible Genetics	1, 14, 30, 50, 73, 77, 87, 89, 90	9
Human Immunodeficiency Virus 1, HIV-1	Diagnosis, prognosis, treatment decisions	RT, PCR, sequencing	Genotype	Abbott	84	1
Human Immunodeficiency Virus 1, Ultrasensitive	Diagnosis, prognosis	RT, PCR, HYB	Quant	Roche	1, 6, 10, 14, 17, 22, 29, 30, 31, 33, 39, 46, 50, 52, 58, 66, 70, 73, 74, 75, 76, 77, 78, 82, 87, 90, 91, 92, 93	29
Human Immunodeficiency Virus, drug resistance	Diagnosis, prognosis, treatment decisions	RT, PCR, sequencing	Genotype	Bayer	33	1
Human Papillomavirus (HPV)	Diagnosis, prognosis	PCR, LiPA	Genotype	Bayer	58	1
Human Papillomavirus (HPV)	Diagnosis, prognosis	Direct Probe	Qual	Chemicon	1, 31, 92	3
Human Papillomavirus (HPV)	Diagnosis, prognosis	PCR, HYB	Qual	Digene	2, 4, 5, 6, 13, 28, 39, 53, 66, 73, 77, 80, 84	13
Human Papillomavirus (HPV)	Diagnosis, prognosis	PCR, LiPA	Qual	Roche	28	1
Human papillomavirus, High Risk Only HPV	Diagnosis	Real-time PCR	Genotype	Third Wave	57	1

Infectious Agent	Test Use	Method	Assay Type	Source of Kits	Laboratory*	Number
Influenza A & B Respiratory Virus	Diagnosis	PCR, HYB	Qual	Flu Vision	77	1
Norwalk virus	Diagnosis	Real-time PCR	Genotype	Cepheid	79	1
RSV, influ A, parainflu123 Influenza A Respiratory virus	Diagnosis	PCR	Qual	Prodessa	6	1
Varicella zoster virus VZV	Diagnosis	PCR, HYB	Qual	Argene Biosoft	50	1

* Refer to Table 12 for Identification Number of Laboratory.

Table 21. Tests Using Commercial Kits: Parasitic or Fungal Infectious Diseases

Infectious Agent	Test Use	Method	Assay Type	Source	Laboratory*	Number
Aspergillis, screening	Diagnosis	NASBA	Qual	bioMerieux	50	1
Blastomyces dermatitidis	Diagnosis, prognosis	Probe hybridization	Qual	Genprobe	6, 77	2
Coccidioides immitis	Diagnosis, prognosis	Probe hybridization	Qual	Genprobe	6, 39, 66, 77	4
Histoplasma capsulatum	Diagnosis, prognosis	Probe hybridization	Qual	Genprobe	6, 39, 77	3
Yeast, molecular typing	Diagnosis, prognosis	PFGE	Qual, Quant	Bio-Rad	20, 39	2

* Refer to Table 12 for Identification Number of Laboratory.

Table 22. Tests Using Commercial Kits: Solid Tumors

Gene	Tumor Type/Subtype	Test Use	Test Method	Assay Type	Laboratory*	Number of Laboratories
ALK	Inflammatory myofibroblastic tumor	Monitoring	FISH	Qual	55, 40, 45, 65, 79	5
Bcl-2	All tumor types	Diagnosis, Prognosis	Immunohistochemistry	Quant	68	1
Beta-catenin	All tumor types	Prognosis	Immunohistochemistry	Quant	68	1
BRAF	Colon cancer	Diagnosis	Real-time PCR	Qual	35	1
Carbonic Anyhydrase	All tumor types	Diagnosis, Prognosis	Immunohistochemistry	Quant	68	1
Caveolin-1	All tumor types	Diagnosis, Prognosis	Immunohistochemistry	Quant	68	1
CD24/CD31/CD44	All tumor types	Diagnosis, Prognosis	Immunohistochemistry	Quant	68	1
Cleaved Caspase 3	All tumor types	Diagnosis, Prognosis	Immunohistochemistry	Quant	68	1
C-kit	Gastrointestinal stromal tumor	Diagnosis	PCR-sequencing, real-time PCR, other	Qual	68, 4	2
c-MET	All tumor types	Diagnosis, Prognosis	Immunohistochemistry	Quant	68	1
C-MYC	Lung cancer, small cell carcinoma, breast cancer, Burkitt/Burkitt-like lymphoma	Monitoring	FISH	Quant, Qual	40, 42, 43, 65, 79, 68	6
COX-2	All tumor types	Diagnosis, Prognosis	Immunohistochemistry	Qual	68	1
cyclin D1/CCND1/PRAD1	Head and neck cancer, squamous cell cancer, breast cancer	Diagnosis, prognosis	FISH	Quant	42, 45	2
DCC	Colorectal cancer	Prognosis	PCR-Allele specific	Quant	42	1
E-cadherin	Prostate cancer, breast cancer, endometrial cancer, gastric cancer	Diagnosis	Immunohistochemistry	Qual	4	1

Gene	Tumor Type/Subtype	Test Use	Test Method	Assay Type	Laboratory*	Number of Laboratories
EGFR	Head and neck cancer, squamous cell cancer, glioblastoma, all tumor types	Diagnosis, treatment decisions	FISH	Quant, Qual	55, 40, 42, 65, 67, 21, 68, 4	8
EpCAM	All tumor types	Diagnosis, Prognosis	Immunohistochemistry	Quant	68	1
ER	Breast cancer	Diagnosis, Prognosis	Immunohistochemistry	Quant	68	1
EWS-ATF1	Sarcomas, clear cell sarcoma	Diagnosis	FISH	Quant, Qual	43, 54	2
EWS-CHN/TEC/PSCTK4	Sarcomas, extraskeletal myxoid chondrosarcoma	Diagnosis	FISH	Quant, Qual	43, 54	2
EWS-CHOP	Sarcomas, myxoid liposarcoma	Diagnosis	FISH	Quant	43	1
EWS-EIAF	Sarcomas, Ewing's sarcoma	Diagnosis	PCR-sequencing	Qual	9	1
EWS-ERG	Sarcomas, Ewing's sarcoma	Diagnosis	FISH	Quant	43	1
EWS-ETV1	Sarcomas, Ewing's sarcoma	Diagnosis	FISH	Quant	43	1
EWS-FEV	Sarcomas, Ewing's sarcoma	Diagnosis	FISH	Quant	43	1
EWS-WT1	Sarcomas, desmoplastic small round cell tumor	Diagnosis	FISH	Quant, Qual	43, 54	2
HER2/neu/ERBB2	Breast cancer, endometrial cancer, uterine serous type	Diagnosis, prognosis	FISH, Immunohistochemistry	Quant, Qual	4, 32, 55, 40, 42, 43, 54, 57, 63, 65, 67, 79, 94, 68	14
IGH/BCL2	Lymphoma	Monitoring	FISH	Qual	40, 45, 65, 79	4
IGH/CCND1	Mantle cell lymphoma	Monitoring	FISH	Qual	79	1
IGH/MYC	Burkitt/Burkitt-like lymphoma	Monitoring	FISH	Qual	79	1
Ki-67	Breast cancer	Diagnosis, Prognosis	Immunohistochemistry	Quant	68	1

Gene	Tumor Type/Subtype	Test Use	Test Method	Assay Type	Laboratory*	Number of Laboratories
KRAS/Ki-Ras	Lung cancer, non-small cell, endometrial cancer, pancreatic cancer, colorectal cancer	Diagnosis	PCR-sequencing, PCR-SSCP	Qual	35	1
KRAS2/Ki-Ras	Colorectal cancer, pancreatic cancer	Diagnosis, treatment decisions	PCR-SSCP	Qual	56	1
MALT	*MALT* lymphomas	Monitoring	FISH	Qual	55, 40, 65, 79	4
mismatch repair genes	Endometrial cancer, colorectal cancer, HNPCC/Lynch syndrome	Diagnosis	FISH, Other	Qual	6, 7, 40	3
MLH1	Colorectal cancer, HNPCC/Lynch syndrome, endometrial cancer	Diagnosis	Real-time PCR, Other	Quant, Qual	4, 37, 48, 15, 21	5
MSH2	Ovarian cancer, colorectal cancer, HNPCC/Lynch syndrome, endometrial cancer	Diagnosis	Southern Blot, Other	Qual	4, 37, 48, 15, 21	5
MSH6/GTBP	Colorectal cancer, HNPCC/Lynch syndrome, endometrial Cancer	Monitoring	Other	Qual	37, 48, 15, 21	4
N-myc/MYCN	Neuroblastoma	Diagnosis, Prognosis	FISH	Qual, Quant	42, 79	2
Not specified	Bladder cancer	Monitoring	FISH	Quant, Qual	42, 79	2
PAX3-FKHR	Sarcomas, alveolar rhabdomyosarcoma	Diagnosis	Real-time PCR, PCR sequencing, PCR-Allele specific, PCR-restriction digest, PCR-ASO blot	Qual, Quant	15	1
PMS2	Colorectal cancer, HNPCC/Lynch syndrome, endometrial Cancer	Diagnosis	Other	Quant	37, 48	2
PRSS1	Pancreatic cancer, hereditary pancreatitis	Diagnosis	PCR sequencing	Qual	3	1
PTEN	Astrocytoma	Diagnosis	FISH	Quant	35	1
p16/CDKN2A/MTS1	Melanoma, bladder cancer, pancreatic cancer	Diagnosis	FISH	Quant, Qual	42, 79, 48, 15, 68	5

Gene	Tumor Type/Subtype	Test Use	Test Method	Assay Type	Laboratory*	Number of Laboratories
p16, 3cen, 7cen	Urothelial cancer	Diagnosis	FISH	Qual	43	1
p53	All tumor types	Diagnosis, Prognosis	Immunohistochemistry	Quant	68	1
Phospho AKT	All tumor types	Diagnosis, Prognosis	Immunohistochemistry	Quant	68	1
PR	Breast cancer	Diagnosis, Prognosis	Immunohistochemistry	Quant	68	1
PSA	Prostate Cancer	Diagnosis	Immunohistochemistry	Qual	4	1
RB1	Retinoblastoma	Diagnosis	FISH	Qual	79	1
RET proto-oncogene	MEN 2A, MEN 2B	Diagnosis	PCR sequencing, PCR-restriction digest	Qual	35, 8, 23, 37	4
Thyroglobulin	Thyroid cancer	Diagnosis	Immunohistochemistry	Qual	4	1
Topo II alpha	Breast cancer	Diagnosis, prognosis	FISH	Quant	68	1
21 genes (unspecified)	Breast cancer	Monitoring, prognosis, treatment decisions	Real-time PCR	Quant	36	1
Unknown	Oligodendroglioma, testicular germ cell tumors, seminomas & nonseminomas, glioblastoma, astrocytoma, neuroblastoma	Monitoring	FISH	Quant, Qual	42, 43, 54, 79	4
UGT1A1	Colorectal cancer	Treatment decisions	PCR	Quant	55, 40, 65, 6, 47, 57	6
Various	All tumor types	Monitoring, diagnosis	PCR-allele specific	Qual	42, 4, 44, 79	4
VHL	Various	Diagnosis	Southern blot, PCR sequencing	Qual	8, 25	2

Note: The manufacturer of the commercial kits used in hematology tests were not listed in the AMP database.

* Refer to Table 12 for Identification Number of Laboratory.

Table 23. Tests Using Commercial Kits: Hematopathology

WHO Category	Gene	Gene Specifics	Test Use	Test Method	Assay Type	Laboratory*	Number of Laboratories
Mastocytosis & Myelodysplastic	*BCR-ABL*	Unspecified, *BCR-ABL major/M-bcr/P210, BCR-ABL minor/m-bcr/P190, BCR-ABL micro/mu-bcr/P230*	Diagnosis, prognosis	RT-PCR, FISH	Qual, Quant	80, 30, 40, 55, 65, 15, 44, 46, 54, 72	10
Mastocytosis & Myelodysplastic	*MLL(HRX)*	Unspecified	Diagnosis, prognosis	RT-PCR, FISH	Qual, Quant	80, 40, 55, 65, 15, 44, 46	7
Mastocytosis & Myelodysplastic	not specified	Unspecified	Diagnosis, prognosis	FISH	Quant	29, 46	2
Mastocytosis & Myelodysplastic	*PDGFRB-TEL(ETV6)*	Unspecified	Diagnosis, prognosis	RT-PCR	Qual	80	1
Acute Myeloid Leukemias	*AF9-MLL(HRX)*	Unspecified	Diagnosis, prognosis	RT-PCR, FISH	Qual, Quant	80, 40, 55, 65, 15, 43	6
Acute Myeloid Leukemias	*AML 1 (RUNX1)(CBFa)/ETO*	Unspecified	Diagnosis, prognosis	FISH	Quant	43, 42, 54	3
Acute Myeloid Leukemias	*BCR-ABL*	Unspecified, *BCR-ABL* breakpoint not specified, *BCR-ABL micro/mu-bcr/P230, BCR-ABL minor/m-bcr/P190, BCR-ABL major/M-bcr/P210*	Diagnosis, prognosis, monitoring	FISH, real-time PCR, RT-PCR, antibody, Southern	Quant, Qual	29, 30, 42, 80, 40, 55, 65, 15, 7, 43, 44, 54, 63, 72	14
Acute Myeloid Leukemias	*CBFb/MYH11*	Unspecified	Diagnosis, prognosis, monitoring	FISH, RT-PCR, real-time PCR	Quant, Qual	29, 42, 80, 55, 15, 43, 54, 18	8
Acute Myeloid Leukemias	*DEK-CAN*	Unspecified	Diagnosis, prognosis	RT-PCR	Qual	80	1
Acute Myeloid Leukemias	*ETO(CBFA2T1)-AML1(RUNX1orCBFA2)*	Unspecified	Diagnosis, prognosis	FISH, real-time PCR, RT-PCR	Quant, Qual	29, 42, 46, 80, 55, 65, 15, 43, 42, 54	10

WHO Category	Gene	Gene Specifics	Test Use	Test Method	Assay Type	Laboratory*	Number of Laboratories
Acute Myeloid Leukemias	Ig Heavy/Light	Ig heavy chain/IgH/14q32, unspecified, Ig light chain/IgK/2p12, Ig light chain/IgL/22q11.2	Diagnosis, prognosis, monitoring	PCR, FISH	Qual, Quant	9, 30, 42, 90, 40, 43, 44, 63, 72	9
Acute Myeloid Leukemias	*MLL(HRX)*	Unspecified	Diagnosis, Prognosis	FISH, RT-PCR	Quant, Qual	9, 29, 80, 81, 40, 55, 65, 15, 42, 43, 54	11
Acute Myeloid Leukemias	*MLL(HRX)-ELL*	Unspecified	Diagnosis, prognosis	RT-PCR, FISH	Qual, Quant	80, 40, 55, 65, 15, 43	6
Acute Myeloid Leukemias	*MLL(HRX)-ENL*	Unspecified	Diagnosis, prognosis	RT-PCR, FISH	Qual, Quant	80, 40, 55, 65, 15, 43	6
Acute Myeloid Leukemias	Not specified	Unspecified	Prognosis	PCR, RT-PCR, antibody	Qual	42, 80, 7	3
Acute Myeloid Leukemias	*NPM-RARa*	Unspecified	Diagnosis, prognosis	RT-PCR, FISH	Qual, Quant	80, 43	2
Acute Myeloid Leukemias	*NUMA-RARa*	Unspecified	Diagnosis, prognosis	FISH	Quant	43	1
Acute Myeloid Leukemias	*PLZF-RARa*	Unspecified	Diagnosis, prognosis	RT-PCR, FISH	Qual, Quant	80, 43	2
Acute Myeloid Leukemias	*PML-RARa*	Unspecified	Diagnosis, prognosis	FISH, RT-PCR	Quant, Qual	29, 80, 40, 55, 15, 42, 43, 54	8
Acute Myeloid Leukemias	*STAT5B-RARa*	Unspecified	Diagnosis, prognosis	FISH	Quant	43	1
Acute Myeloid Leukemias	*TCR* genes	*TCR* gamma/7p15, unspecified	Diagnosis, prognosis, monitoring	PCR	Qual	9, 30, 43, 90, 40, 55, 65, 42, 72	9
Acute Myeloid Leukemias	*TEL(ETV6)*	Unspecified	Diagnosis, prognosis	RT-PCR, FISH	Qual	80, 65, 55, 42	4
B and T Cell Neoplasms	*ATM,* ?other genes	Unspecified	Diagnosis, prognosis	FISH	Quant	29, 21, 42, 43	4

WHO Category	Gene	Gene Specifics	Test Use	Test Method	Assay Type	Laboratory*	Number of Laboratories
B and T Cell Neoplasms	*BCL-1*	Unspecified	Diagnosis, prognosis	FISH	Quant	43	1
B and T Cell Neoplasms	*BCL-1 (PRAD1 or CCND1 or cyclin D1)*	*BCL-1* unspecified breakpoint, unspecified	Diagnosis, prognosis	FISH	Qual, Quant	29, 40, 55, 65, 15, 43, 54	7
B and T Cell Neoplasms	*BCL-1-IgH*	Unspecified	Diagnosis, prognosis	FISH	Quant, Qual	29, 40, 55, 65, 43, 54	6
B and T Cell Neoplasms	*BCL-2*	BCL-2 unspecified breakpoint, *BCL-2 mcr, BCL-2 MBR*, unspecified	Diagnosis, prognosis, monitoring	FISH, PCR, real-time PCR	Qual, Quant	29, 30, 42, 61, 40, 55, 65, 43, 54	9
B and T Cell Neoplasms	*BCL-3*	*BCL-2 MBR*	Prognosis	PCR	Qual	30	1
B and T Cell Neoplasms	*BCL-6(LAZ3)*	Unspecified	Diagnosis, prognosis	FISH	Qual, Quant	29, 65, 42, 43, 54	5
B and T Cell Neoplasms	*BCR-ABL*	Unspecified, *BCR-ABL* breakpoint not specified, *BCR-ABL micro/mu-bcr/P230, BCR-ABL minor/m-bcr/P190, BCR-ABL major/M-bcr/P210*	Diagnosis, prognosis, monitoring	Real-time PCR, FISH, RT-PCR	Quant	30, 80, 40, 15, 43, 44, 72	7
B and T Cell Neoplasms	*BIRC3(API2)-MALT1(MLT)*	Unspecified	Diagnosis, prognosis	FISH	Quant, Qual	54, 43	2
B and T Cell Neoplasms	*EBV*	Unspecified	Diagnosis, prognosis	Real-time PCR	Quant	29, 40, 55, 65	4
B and T Cell Neoplasms	Ig Heavy/Light	Ig heavy chain/IgH/14q32, Ig (unspecified gene), Ig light chain/IgK/2p12, Ig light chain/IgL/22q11.2, unspecified	Diagnosis, prognosis, monitoring	PCR, PCR sequencing, FISH	Qual, Quant	9, 29, 30, 42, 90, 40, 43, 44, 54, 72	10
B and T Cell Neoplasms	*MLL(HRX)*	Unspecified	Diagnosis, prognosis	RT-PCR, FISH	Qual, Quant	80, 40, 55, 65, 15, 43	6
B and T Cell Neoplasms	*MYC*	Unspecified	Diagnosis, prognosis	FISH	Quant, Qual	42, 43, 54	3

WHO Category	Gene	Gene Specifics	Test Use	Test Method	Assay Type	Laboratory*	Number of Laboratories
B and T Cell Neoplasms	*MYC-IgH* and variants	Unspecified	Diagnosis, prognosis	FISH	Qual, Quant	29, 40, 55, 65, 15, 43, 54	7
B and T Cell Neoplasms	Not specified	Unspecified	Diagnosis, prognosis	FISH	Quant	29, 42, 43	3
B and T Cell Neoplasms	*NPM-ALK*	Unspecified	Diagnosis, prognosis	PCR, FISH	Quant, Qual	42, 5, 43	3
B and T Cell Neoplasms	*NPM1-ALK* or other *ALK*	Unspecified	Diagnosis, prognosis	FISH	Qual	29, 40, 55, 65, 15, 43	6
B and T Cell Neoplasms	*P53*	Unspecified	Diagnosis, prognosis	FISH	Quant	29, 40, 55, 65, 56, 21, 15, 42, 43	9
B and T Cell Neoplasms	*P53*, other genes?	Unspecified	Diagnosis, prognosis	FISH	Quant	43	1
B and T Cell Neoplasms	*PBX-E2A*	Unspecified	Diagnosis, prognosis	RT-PCR	Qual	80	1
B and T Cell Neoplasms	*TAL1*	Unspecified	Diagnosis, prognosis	RT-PCR	Qual	80	1
B and T Cell Neoplasms	*TCR* genes	TCR gamma/7p15, unspecified	Diagnosis, prognosis, monitoring	PCR	Qual	9, 29, 30, 42, 90, 40, 55, 65, 72	9
B and T Cell Neoplasms	*TEL(ETV6)/AML1*	Unspecified	Diagnosis, prognosis	FISH, RT-PCR	Quant, Qual	42, 43, 80	3
B and T Cell Neoplasms	Various	Unspecified	Diagnosis, prognosis	Antibody	Qual	74	1
Chronic Myeloproliferative Diseases	*BCR-ABL*	*BCR-ABL* breakpoint not specified, unspecified, *BCR-ABL micro/mu-bcr/P230, BCR-ABL minor/m-bcr/P190, BCR-ABL major/M-bcr/P210*	Diagnosis, prognosis, monitoring	FISH, real-time PCR, RT-PCR	Quant, Qual	29, 30, 42, 46, 80, 40, 55, 65, 15, 54, 72	11

WHO Category	Gene	Gene Specifics	Test Use	Test Method	Assay Type	Laboratory*	Number of Laboratories
Chronic Myeloproliferative Diseases	*PDGFRB-TEL(ETV6)*	unspecified	Diagnosis, prognosis	RT-PCR	Qual	80	1
Histiocytic and Dendritic-Cell Neoplasms	Ig Heavy/Light	Ig heavy chain/IgH/14q32, unspecified	Diagnosis, prognosis	PCR	Qual	30, 90, 40	3
Histiocytic and Dendritic-Cell Neoplasms	*TCR* genes	Unspecified	Diagnosis, prognosis	PCR, PCR and mutation scanning	Qual	30, 90, 40, 55, 65	5
Hodgkin Lymphoma	Ig Heavy/Light	Ig heavy chain/IgH/14q32, unspecified, Ig light chain/Igk/2p12	Diagnosis, prognosis	PCR	Qual	9, 30, 90, 40, 44	5

Note: The manufacturer of the commercial kits used in hematology tests were not listed in the AMP database.

* Refer to Table 12 for Identification Number of Laboratory.

FDA-approved Molecular Test Tables

Table 24 through Table 28 presents a listing of the *in vitro* molecular diagnostic products that are approved or cleared for diagnostic use in the United States by the Food and Drug Administration (FDA). Molecular tests are classified as "Medical Devices." The information presented in the tables is current through December 10, 2009. Further information about the products presented in each table is available at the Web site for FDA's Office of *In Vitro* Diagnostic (OIVD) Evaluation and Safety, http://www.fda.gov/cdrh/oivd/index.html.

Table 24. FDA-approved or cleared Molecular Diagnostic Tests: Bacterial and Fungal Infectious Diseases

Test Use	Test Name	Manufacturer	Test Method
Bacillus anthracis	Joint Biological Agent Identification and Diagnostic System (JBAIDS) Anthrax Detection kit	Idaho Technology, Inc. Salt Lake City, UT	Real-time PCR
Candida albicans and Candida spp.	*C. albicans* PNA FISH	AdvanDx Woburn, MA	PNA FISH
	Yeast Traffic Light PNA FISH™[1]	AdvanDx Woburn, MA	PNA FISH
Clostridium difficile	GeneOhm Cdiff Assay	BD Diagnostics—GeneOhm San Diego, CA	Real-time PCR
	Xpert™ C. difficile Test	Cepheid Sunnyvale, CA	Real-time PCR
	ProGastro™ Cd Assay	Prodesse Waukesha, WI	Multiplex Real-time PCR
Chlamydia trachomatis detection (single organism)	BD ProbeTec™ Chlamydia trachomatis (CT) Q^x Amplified DNA Assay	BD Diagnostic Systems Sparks, MD	SDA
	HC2® CT ID	Qiagen Germantown, MD	Hybrid Capture
	APTIMA CT® Assay	Gen-Probe, Inc. San Diego, CA	TC, TMA, DKA
	PACE® 2 CT Probe Competition Assay (Ct-confirmation test)	Gen-Probe, Inc. San Diego, CA	HPA
	AMPLICOR® CT/NG Test for *Chlamydia trachomatis*	Roche Molecular Diagnostics Pleasanton, CA	PCR
	COBAS AMPLICOR® CT/NG Test for *Chlamydia trachomatis*[1]	Roche Molecular Diagnostics Pleasanton, CA	PCR

Test Use	Test Name	Manufacturer	Test Method
Neisseria gonorrhoeae detection (single organism)	BD ProbeTec™ Neisseria gonorrhoeae (GC) Q^X Amplified DNA Assay	BD Diagnostic Systems Sparks, MD	SDA
	HC2® GC ID	Qiagen Germantown, MD	Hybrid Capture
	APTIMA® GC Assay	Gen-Probe, Inc. San Diego, CA	TC, TMA, DKA
	PACE® 2 GC Probe Competition Assay (GC-confirmation test)	Gen-Probe, Inc. San Diego, CA	HPA
	AMPLICOR® CT/NG Test for *Neisseria gonorrhoeae*	Roche Molecular Diagnostics Pleasanton, CA	PCR
	COBAS AMPLICOR® CT/NG Test for *Neisseria gonorrhoeae*[1]	Roche Molecular Diagnostics Pleasanton, CA	PCR
Chlamydia trachomatis and *Neisseria gonorrhoeae* detection	Abbott Molecular, Inc. Des Plaines, IL	Abbott® Real Time CT/NG	Real-time PCR
	BD ProbeTec™ ET *C. trachomatis* and *N. gonorrhoeae* amplified DNA Assay	BD Diagnostics—GeneOhm San Diego, CA	SDA
	HC2® CT/GC Combo Test	Qiagen Germantown, MD	Hybrid Capture
	APTIMA Combo 2® Assay	Gen-Probe, Inc. San Diego, CA	TC, TMA, DKA
	PACE® 2C CT/GC	Gen-Probe, Inc. San Diego, CA	HPA
	AMPLICOR® CT/NG Test	Roche Molecular Diagnostics Pleasanton, CA	PCR
	COBAS AMPLICOR™ CT/NG Test[1]	Roche Molecular Diagnostics Pleasanton, CA	PCR
Enterococcus faecalis	*Enterococcus faecalis* PNA FISH	AdvanDx Woburn, MA	PNA FISH
Escherichia coli and Pseudomonas aeruginosa	*E. coli/P. aeruginosa PNA FISH*	AdvanDx Woburn, MA	PNA FISH
Escherichia coli, Klebsiella pneumonia and Pseudomonas aeruginosa	*EK/P. aeruginosa PNA FISH*	AdvanDx Woburn, MA	PNA FISH
Francisella tularensis	Joint Biological Agent Identification and Diagnostic System (JBAIDS) Tularemia Detection kit	Idaho Technology, Inc. Salt Lake City, UT	Real-time PCR
Gardnerella, Trichomonas vaginalis and *Candida spp.* detection	BD Affirm™ VPIII Microbial Identification Test	BD Diagnostics-GeneOhm San Diego, CA	Hybridization

Test Use	Test Name	Manufacturer	Test Method
Group A *Streptococci* detection	Group A Strep direct (GASD)	Gen-Probe, Inc. San Diego, CA	HPA
Group B *Streptococci* detection	GBS PNA FISH	AdvanDx Woburn, MA	PNA FISH
	IDI-Strep B™ Assay	BD Diagnostics—GeneOhm San Diego, CA	Real-time PCR
	Smart GBS	Cepheid Sunnyvale, CA	Real-time PCR
	Xpert™ GBS	Cepheid Sunnyvale, CA	Real-time PCR
	Group B AccuProbe®	Gen-Probe, Inc. San Diego, CA	HPA
	IDI-Strep B™ Assay	Becton, Dickinson & Company Sparks, MD	Real-time PCR
MRSA for *Staphylococcus aureus* Screening assay	IDI-MRSA™ Assay	BD Diagnostics—GeneOhm San Diego, CA	Real-time PCR
	Xpert™ MRSA	Cepheid Sunnyvale, CA	Real-time PCR
MRSA for *Staphylococcus aureus* Diagnostic assay for positive blood cultures	GeneOhm StaphSR	BD Diagnostics—GeneOhm San Diego, CA	Real-time PCR
	Xpert™ MRSA/SA Blood Culture Assay	Cepheid Sunnyvale, CA	Real-time PCR
MRSA for *Staphylococcus aureus* Diagnostic assay for Skin and Soft Tissue Infection	Xpert(TM) MRSA/SA Skin and Soft Tissue Infection (SSTI) test	Cepheid Sunnyvale, CA	Real-time PCR
Mycobacterium tuberculosis detection	AMPLIFIED™ *Mycobacterium tuberculosis* Direct Test (MTD)	Gen-Probe, Inc. San Diego, CA	TMA
	AMPLICOR™ *Mycobacterium tuberculosis* Test	Roche Molecular Diagnostics Pleasanton, CA	PCR
Mycobacteria spp., different fungi and bacteria culture confirmation[2]	AccuProbe® Culture Identification Tests	Gen-Probe, Inc. San Diego, CA	HPA
Staphylococcus aureus	*S. aureus* PNA FISH	AdvanDx Woburn, MA	PNA FISH

[1] Five Candida species directly from positive blood cultures including: C. albicans and/or C. parapsilosis, C. tropicalis, and C. glabrata and/or C. krusei

[2] C. trachomatis and N. gonorrhoeae detection may now be done using the Roche COBAS Amplicor system directly from Cytyc Corporation's ThinPrep Pap test collection kit; this use is FDA Approved.

[3] Campylobacter spp., Enterococcus spp., Group B Streptococcus, Haemophilus influenzae, Neisseria gonorrhoeae, Streptococcus pneumoniae, Staphylococcus aureus, Listeria monocytogenes, Group A Streptococcus, Mycobacterium avium, Mycobacterium intracellulare, Mycobacterium avium complex, Mycobacterium gordonae, Mycobacterium tuberculosis complex, Mycobacterium kansasii, Blastomyces dermatitidis, Coccidioides immitis, Crytococcus neoformans, Histoplasma capsulatum.

Table 25. FDA-approved or cleared Molecular Diagnostic Tests: Viral Infectious Disease

Test Use	Test Name	Manufacturer	Test Method
Avian Flu diagnosis	Influenza A/H5	Centers for Disease Control and Prevention	Real-time RT-PCR
Cytomegalovirus detection	HC1® CMV DNA Test	Qiagen Germantown, MD	Hybrid Capture
	Hybrid Capture® CMV DNA Test	Gentech Diagnostics Pvt. Ltd. New Delhi, India	Hybrid Capture with Chemi-luminescent
	CMV pp67 mRNA	bioMerieux, Inc. Durham, NC	NASBA
Enterovirus detection	NucliSENS EasyQ® Enterovirus (also see under *Systems* below: NucliSENS EasyQ® System)	bioMérieux, Inc. Durham, NC	Real-time NASBA
	Xpert™ EV	Cepheid Sunnyvale, CA	Real-time PCR
HBV quantitation	COBAS® TaqMan® HBV Test, for use with the HighPure System	Roche Molecular Diagnostics Pleasanton, CA	PCR
HCV qualitative detection	VERSANT® HCV RNA	Gen-Probe, Inc. San Diego, CA (distributed by Bayer HealthCare, Berkeley, CA)	TMA
	AMPLICOR™ HCV Test, v2.0	Roche Molecular Diagnostics Pleasanton, CA	PCR
	COBAS AMPLICOR™ HCV Test, v2.0	Roche Molecular Diagnostics Pleasanton, CA	PCR
HCV quantitation	VERSANT® HCV RNA 3.0 Assay (bDNA)	Siemens Healthcare Diagnostics Deerfield, IL	bDNA
	COBAS® AmpliPrep/COBAS® TaqMan HCV Test	Roche Molecular Diagnostics Pleasanton, CA	Real-time RT-PCR
HIV drug resistance testing	ViroSeq™ HIV-1 Genotyping System	Celera Diagnostics Alameda, CA (distributed by Abbott Laboratories, Abbott Park, Il)	Sequencing
	TruGene™ HIV-1 Genotyping and Open Gene DNA Sequencing System	Siemens Healthcare Diagnostics Deerfield, IL	Sequencing

Test Use	Test Name	Manufacturer	Test Method
HIV quantitation	Abbott Real-time HIV-1	Abbott Molecular, Inc. Des Plaines, IL	Real-time RT-PCR
	VERSANT® HIV-1 RNA 3.0 Assay (bDNA)	Siemens Healthcare Diagnostics Deerfield, IL	bDNA
	NucliSens®HIV-1 QT	bioMerieux, Inc. Durham, NC	NASBA
	AMPLICOR HIV-1 MONITOR™ Test, v1.5	Roche Molecular Diagnostics Pleasanton, CA	RT-PCR
	COBAS AMPLICOR HIV-1 MONITOR™ Test, v1.5	Roche Molecular Diagnostics Pleasanton, CA	RT-PCR
	COBAS® AmpliPrep/ COBAS® TaqMan HIV-1 Test	Roche Molecular Diagnostics Pleasanton, CA	RT-PCR
HBV/HCV/HIV for blood donations	Hepatitis C Virus (HCV) Reverse Transcription (RT) Polymerase Chain Reaction (PCR) assay	BioLife Plasma Services, L.P. Deerfield, IL 60015	RT-PCR
	Human Immunodeficiency Virus, Type 1 (HIV-1) Reverse Transcription (RT) Polymerase Chain Reaction (PCR) assay	BioLife Plasma Services, L.P. Deerfield, IL 60015	RT-PCR
	Procleix™ HIV-1/HCV Assay	Gen-Probe, Inc. San Diego, CA (distributed by Chiron)	TC, TMA, HPA
	PROCLEIX ULTRIO® Assay (HIV-1, HCV and HBV)	Gen-Probe, Inc. San Diego, CA (distributed by Chiron)	TC, TMA, HPA
	UltraQual™ HCV RT-PCR Assay	National Genetics Institute Los Angeles, CA	RT-PCR
	UltraQual™ HIV-1 RT-PCR Assay	National Genetics Institute Los Angeles, CA	RT-PCR
	COBAS AmpliScreen™ HBV Test	Roche Molecular Diagnostics Pleasanton, CA	PCR
	COBAS AmpliScreen™ HCV Test, v2.0	Roche Molecular Diagnostics Pleasanton, CA	RT-PCR
	COBAS AmpliScreen™ HIV-1 Test, v1.5	Roche Molecular Diagnostics Pleasanton, CA	RT-PCR
	COBAS® TaqScreen MPX®	Roche Molecular Diagnostics Pleasanton, CA	Multiplex Real-time PCR and RT-PCR

Test Use	Test Name	Manufacturer	Test Method
Human Metapneumovirus	Pro hMPV+™ Assay	Prodesse Waukesha, WI	Multiplex Real-time PCR
Human Papillomavirus testing	Cervista™ HPV HR (high risk)	Hologic, Inc. (Third Wave Technologies) Bedford, MA	Invader® Chemistry
	Cervista™ HPV 16/18	Hologic, Inc. (Third Wave Technologies) Bedford, MA	Invader® Chemistry
	HC2® HR and LR	Qiagen Germantown, MD	Hybrid Capture
	HC2®HPV HR	Qiagen Germantown, MD	Hybrid Capture
	HC2® DNA with Pap	Qiagen Germantown, MD	Hybrid Capture
Influenza Virus Panel	Human Influenza Virus Real-time RT-PCR Detection and Characterization Panel (also see under *Systems* below: ABI 7500 Fast Dx Real-Time PCR Instrument)	Centers for Disease Control Atlanta, GA	Real-time RT-PCR
Respiratory virus panel	xTAG Respiratory Viral Panel[1] (also see under *Systems* below: Luminex LX 100/200)	Luminex Molecular Diagnostics Toronto, Canada	PCR, ASPE, Tag sorting
	Verigene® Respiratory Virus Nucleic Acid Test and Verigene® Respiratory Virus Test-SP	Nanosphere, Inc. Northbrook, IL	Multiplex Gold Nanoparticle Probes
	ProFlu+™ Assay[2]	Prodesse Waukesha, WI	Multiplex Real-time PCR
West Nile for blood donations	Procleix WNV	Gen-Probe, Inc. San Diego, CA (distributed by Chiron)	Real-time PCR
	Cobas TaqScreen WNV	Roche Molecular Diagnostics Pleasanton, CA	PCR

Table 26. FDA-approved or cleared Non-Infectious-Disease-Related Molecular Diagnostic Test

Test Use	Test Name	Manufacturer	Test Method
B-Cell chronic lymphocytic leukemia (B-CLL)	CEP®12 DNA Probe Kit	Abbott Molecular, Inc. Des Plaines, IL	FISH
Breast cancer - determination of the likelihood of metastasis	MammaPrint	Agendia, Amsterdam, The Netherlands	Microarray analysis
	GeneSearch ™ BLN Test	Veridex, LLC Warren, NJ	Real-time RT-PCR
Breast Cancer - detection of amplifications and deletions of the *TOP2A* gene	*TOP2A* FISH pharmDx™ Kit	Dako Denmark A/S Glostrup, Denmark DK-2600	FISH on FFPE breast tissue
Chromosome 8 enumeration (CML, AML, MPD, MDS)	CEP®8 DNA Probe Kit	Abbott Molecular, Inc. Des Plaines, IL	FISH
Drug-metabolizing enzymes	AmpliChip™ Cytochrome P450 Genotyping Test	Roche Diagnostics Pleasanton, CA	Microarray
	Verigene® Warfarin Warfarin Metabolism Nucleic Acid Test	Nanosphere, Inc. Northbrook, IL	Multiplex gold nanoparticle probes
	INFINITI™ 2C9 & VKORC1 Multiplex Assay for Warfarin	AutoGenomics, Inc. Carlsbad, CA	PCR and Detection Primer Extension
	eSensor Warfarin Sensitivity Test	Osmetech Molecular Diagnostics Pasadena, CA	PCR, Probe Hybridization
	Gentris Rapid Genotyping Assay -CYP2C9 & VKORC1	ParagonDx, LLC Morrisville, NC	Real-time PCR
	Invader® UGT1A1 Molecular Assay	Hologic, Inc. (Third Wave Technologies) Bedford, MA	Invader® Chemistry
	eQ-PCR™ LC Warfari Genotyping Kit	TrimGen Corp Sparks, MD	Real-time PCR
Factor II (prothrombin)	INFINITI™ System Assay for Factor II	AutoGenomics, Inc. Carlsbad, CA	PCR and detection primer extension
	Factor II (prothrombin) G20210A kit	Roche Diagnostics Pleasanton, CA	Real-time PCR
	Verigene® F2 Nucleic Acid Test	Nanosphere, Inc. Northbrook, IL	Multiplex Gold Nanoparticle Probes
Factor V Leiden	INFINITI™ System Assay for Factor V	AutoGenomics, Inc. Carlsbad, CA	PCR and detection primer extension
	Factor V Leiden kit	Roche Diagnostics Pleasanton, CA	Real-time PCR
	Verigene® F5 Nucleic Acid Test	Nanosphere, Inc. Northbrook, IL	Multiplex gold nanoparticle probes

Test Use	Test Name	Manufacturer	Test Method
Factor II (Prothrombin) and Factor V Leiden G	INFINITI™ System Assay for Factor II & Factor V	AutoGenomics, Inc. Carlsbad, CA	PCR and detection primer extension
	Verigene® F5/F2 Nucleic Acid Test	Nanosphere, Inc. Northbrook, IL	Multiplex gold nanoparticle probes
Factor II (Prothrombin), Factor V Leiden and MTHFR	Verigene® F5/F2/MTHFR Nucleic Acid Test	Nanosphere, Inc. Northbrook, IL	Multiplex gold nanoparticle probes
	INFINITI™ System Assay for Factor II & Factor V	AutoGenomics, Inc. Carlsbad, CA	PCR and detection primer extension
Heart Transplant Recipients - Identification of the potential risk for transplant rejection	AlloMap® Molecular Expression Testing	XDx, Inc. Brisbane, CA	Quantitative Real-time PCR
HER-2 (*ERBB2*) Status	PathVysion® HER-2 DNA Probe kit (also see under *Systems* below: Vysis AutoVysion™ System)	Abbott Molecular, Inc. Des Plaines, IL	FISH
	Her2 FISH pharmDx™ Kit	Dako Denmark A/S Glostrup, Denmark DK-2600	FISH on FFPE breast tissue
	SPOT-Light HER2 CISH kit	Invitrogen Carlsbad, CA	Chromogenic *in situ* hybridization
Initial diagnosis of bladder cancer in patients with hematuria and monitoring for recurrence of bladder cancer	UroVysion®	Abbott Molecular, Inc. Des Plaines, IL	FISH
5,10-methylenetetra hydrofolate reductase (MTHFR)	Verigene® MTHFR Nucleic Acid Test	Nanosphere, Inc. Northbrook, IL	Multiplex gold nanoparticle probes

Table 27. FDA-approved Molecular Diagnostic Control Material

Type of Control	Control Name	Manufacturer	Approved Use
C. trachomatis and *N. gonorrhoeae* controls (positive and negative controls)	Amplichek CT/GC Controls	Bio-Rad Laboratories Irvine, CA	For diagnostic test kits that detect *C trachomatis* and *N. gonorrhoeae* from swabs or urine
C. trachomatis and *N. gonorrhoeae* Positive Quality controls	ACCURUN 341 Chlamydia trachomatis Neisseria gonnorrhoeae DNA Positive Control	SeraCare Life Sciences West Bridgewater, MA	For diagnostic test kits that detect *C. trachomatis* and *N. gonorrhoeae* from swabs or urine
CMV DNA controls	ACCURUN 350 CMV DNA Positive Quality Control	SeraCare Life Sciences West Bridgewater, MA	For *in vitro* tests that detect CMV DNA
Cytochrome P450 2D6 gene (*CYP2D6*)	CYP2D6 *4A/*2AxN	ParagonDx, LLC Morrisville, NC	For diagnostic testing for the Cytochrome P450 2D6 gene variant
	CYP2D6 *2M/*17	ParagonDx, LLC Morrisville, NC	For diagnostic testing for the Cytochrome P450 2D6 gene variant
	CYP2D6 *29/*2AxN	ParagonDx, LLC Morrisville, NC	For diagnostic testing for the Cytochrome P450 2D6 gene variant
	CYP2D6 *6B/*41	ParagonDx, LLC Morrisville, NC	For diagnostic testing for the Cytochrome P450 2D6 gene variant
	CYP2D6 *1/*5	ParagonDx, LLC Morrisville, NC	For diagnostic testing for the Cytochrome P450 2D6 gene variant
	CYP2D6 *3A/*4A	ParagonDx, LLC Morrisville, NC	For diagnostic testing for the Cytochrome P450 2D6 gene variant
HBV controls	OptiQual™ HBV DNA Positive Control	AcroMetrix Benicia, CA	For diagnostic test kits that detect HBV DNA
	VeriSure Pro HBV	AcroMetrix Benicia, CA	Testing in donors
HCV control	OptiQual™ HCV RNA Positive Control	AcroMetrix Benicia, CA	For diagnostic test kits that detect HCV RNA
	VeriSure Pro HCV	AcroMetrix Benicia, CA	Testing in donors
	ACCURUN 305 HCV RNA Positive Control	SeraCare Life Sciences West Bridgewater, MA	For diagnostic test kits that detect HCV RNA
HIV-1 control	OptiQual™ HIV-1 RNA Positive Control	AcroMetrix Benicia, CA	Control ranges to quantitate HIV-1 RNA
	ACCURUN 315 HIV-1 RNA Positive Control	SeraCare Life Science West Bridgewater, MA	Control ranges to quantitate HIV-1 RNA

Type of Control	Control Name	Manufacturer	Approved Use
HIV-1/HCV controls	VeriSure Pro HIV-1	AcroMetrix Benicia, CA	Testing in donors
	Chiron Procleix HIV-1/HCV Controls	Gen-Probe, Inc. San Diego, CA	Testing in donors
HIV-1/HCV Proficiency Panel	Chiron Procleix HIV-1/HCV Proficiency Panel	Gen-Probe, Inc. San Diego, CA	HIV-1/HCV proficiency panel
HIV-1/HCV/HBV controls	VeriSure Pro Negative	AcroMetrix Benicia, CA	Testing in donors
	VeriSure Triplex HIV-1 RNA, HCV RNA, HBV DNA	AcroMetrix Benicia, CA	For diagnostic test kits that detect HIV-1 RNA, HCV RNA and HBV DNA
	ACCURUN 345 HIV-1 RNA, HCV RNA, HBV DNA Positive Quality Control Series 150	SeraCare Life Sciences West Bridgewater, MA	For diagnostic test kits that detect HIV-1 RNA, HCV RNA, and HBV DNA
	ACCURUN 803 Nucleic Acid Negative Quality Control (HIV-1, HCV, HBV)	SeraCare Life Sciences West Bridgewater, MA	For diagnostic test kits that detect HIV-1 RNA, HCV RNA, and HBV DNA
HPV DNA controls	ACCURUN 370 HPV DNA Positive Quality Control	SeraCare Life Sciences West Bridgewater, MA	For *in vitro* tests that detect HPV DNA in human cervical samples
	ACCURUN 870 HPV DNA Negative Quality Control	SeraCare Life Sciences West Bridgewater, MA	For *in vitro* tests that detect HPV DNA in human cervical samples
Human genomic DNA control	Gentrisure™ Human Genomic DNA Reference Control	ParagonDx, LLC Morrisville, NC	For quality control of human DNA tests
West Nile virus RNA controls	ACCURUN 365 West Nile Virus Positive quality Control	SeraCare Life Sciences West Bridgewater, MA	For *in vitro* tests that detect West Nile RNA in human plasma from blood donors
	ACCURUN 865 West Nile Virus Negative quality Control	SeraCare Life Sciences West Bridgewater, MA	For *in vitro* tests that detect West Nile RNA in human plasma from blood donors
	VeriSure Pro WNV External Controls	AcroMetrix Benicia, CA	For use with the Procleix WNV Assay to detect WNV RNA in human plasma from blood donors

Table 28. FDA-approved Molecular Diagnostic Systems

Type of System	System Name	Manufacturer	Approved Use
Amplified molecular diagnostic testing instruments	NucliSENS EasyQ® System	bioMérieux, Inc. Durham, NC	NucliSENS EasyQ® Enterovirus
	BD Viper™System	Becton, Dickinson & Company Sparks, MD	*Chlamydia trachomatis* and *Neisseria gonorrhoeae*
	TIGRIS® DTS™ System	Gen-Probe, Inc. San Diego, CA	Gen-Probe's APTIMA Combo 2® Ct/Ng Assay
	Procleix® Semi-Automated Instrument System	Gen-Probe, Inc. San Diego, CA	For Donated Blood with the PROCLEX® HIV-1/HCV Assay, the PROCLEIX ULTRIO® Assay (HIV-1, HCV and HBV)
	Procleix® TIGRIS Instrument System	Gen-Probe, Inc. San Diego, CA	For Donated Blood with the PROCLEX® HIV-1/HCV Assay, the PROCLEIX ULTRIO® Assay (HIV-1, HCV and HBV) and Procleix® WNV Assay
	VERSANT™ 440 Molecular System	Siemens Healthcare Diagnostic Deerfield, IL	VERSANT HCV RNA 3.0 assay
Extraction Systems	QIAcube	Qiagen, Inc. Germantown, MD	PAXgene Blood RNA System
Microarray systems and high multiplex systems	Affymetrix GCS 3000Dx Instrumentation System	Affymetrix, Inc. Santa Clara, CA and Roche Diagnostics Pleasanton, CA	AmpliChip Cytochrome P450 Genotyping Test
	INFINITI™ Analyzer	AutoGenomics, Inc. Carlsbad, CA	Factor II (Prothrombin) G20210G and Factor V Leiden, and Warfarin G1691A
	Luminex LX 100/200	Luminex Corp. Toronto, Canada	ID-Tag™ respiratory virus panel
	Verigene® System	Nanosphere, Inc. Northbrook, IL	Verigene® Factor V, Factor II, MTHFR and Warfarin
	eSensor® XT-8 System	Osmetech Molecular Diagnostics Pasadena, CA	CFTR and Warfarin

Type of System	System Name	Manufacturer	Approved Use
Real-time PCR amplification systems	Abbott m2000™ (m2000sp + m2000rt)	Abbott Molecular, Inc. Des Plaines, IL	Abbott Real-time HIV-1
	7500 Fast Dx Real-Time PCR Instrument	Applied Biosystems, Inc Foster City, CA	Human Influenza Virus Real-Time RT-PCR Detection and Characterization Panel
	LightCycler Instrument v. 1.2	Roche Diagnostics Pleasanton, CA	Factor II (prothrombin) G20210A kit and Factor V Leiden kit
	COBAS Taqman™ Analyzer	Roche Diagnostics Pleasanton, CA	COBAS AmpliPrep™ System, HIV, HCV and HBV
	Vysis AutoVysion™ System	Abbott Molecular, Inc. Des Plaines, IL	Vysis PathVysion® HER-2 DNA Probe Kit
FISH scanning platform	BioView Duet Scanning System	BioView, Ltd. Nes Ziona, Israel	Peripheral blood and bone marrow (hematological probes), amniotic fluids (x,y,18, 13, and 21 probes), urine (Vysis UroVysion probe)

Appendix C. Evidence Tables for Chapter 3: Clinical Validity and Utility

Table 29. Quality of Systematic Reviews

Checkpoint	Was an 'a priori' design provided?	Was there duplicate study selection and abstraction?	Was a comprehensive literature search performed?	Was the status of publication (i.e., English only) used as inclusion criterion?	Was a list of studies (included and excluded) provided?	Were the characteristics of the included studies assessed and documented?	Was the scientific quality of the included studies assessed and documented?	Was the scientific quality of the included studies used appropriately in formulating the conclusions?	Were methods used to combine the findings of studies appropriate?	Was the likelihood of publication bias assessed?	Was any conflict of interest stated?	Overall Rating
Blue Cross and Blue Shield Association 2008[80]	Yes	CA	Yes	CA	No	Yes	Yes	Yes	Yes	CA	Yes	Moderate
Blue Cross and Blue Shield Association 2008[81]	Yes	CA	Yes	CA	No	Yes	Yes	Yes	Yes	CA	Yes	Moderate
Blue Cross and Blue Shield Association 2008[82]	Yes	CA	Yes	Yes	No	Yes	Yes	Yes	Yes	CA	Yes	High
Marchionni et al. 2008[38]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	High
National Institute for Health Research 2007[69]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	High

Checkpoint	Was an 'a priori' design provided?	Was there duplicate study selection and abstraction?	Was a comprehensive literature search performed?	Was the status of publication (i.e., English only) used as inclusion criterion?	Was a list of studies (included and excluded) provided?	Were the characteristics of the included studies assessed and documented?	Was the scientific quality of the included studies assessed and documented?	Was the scientific quality of the included studies used appropriately in formulating the conclusions?	Were methods used to combine the findings of studies appropriate?	Was the likelihood of publication bias assessed?	Was any conflict of interest stated?	Overall Rating
Dendukuri et al. 2007[72]	CA	CA	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	High
Mocellin et al. 2007[71]	CA	CA	Yes	CA	No	Yes	Yes	Yes	Yes	Yes	Yes	Moderate
Greco et al. 2006[64]	CA	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	High
Lyman & Kuderer 2006[43]	CA	Yes	Yes	No	No	Yes	CA	CA	Yes	Yes	No	Moderate
Matcher et al. 2006[37]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	CA	CA	Yes	High
Flores et al. 2005[66]	CA	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	High
Morgan et al. 2005[65]	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	High
Sanderson et al. 2005[83]	CA	Yes	Yes	CA	No	Yes	Yes	Yes	Yes	No	No	Moderate

Checkpoint	Was an 'a priori' design provided?	Was there duplicate study selection and abstraction?	Was a comprehensive literature search performed?	Was the status of publication (i.e., English only) used as inclusion criterion?	Was a list of studies (included and excluded) provided?	Were the characteristics of the included studies assessed and documented?	Was the scientific quality of the included studies assessed and documented?	Was the scientific quality of the included studies used appropriately in formulating the conclusions?	Were methods used to combine the findings of studies appropriate?	Was the likelihood of publication bias assessed?	Was any conflict of interest stated?	Overall Rating
Pakos et al. 2004[73]	CA	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	No	Moderate
Pai et al. 2004[56]	CA	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	High
Paraskevaidis et al. 2004[75]	No	CA	Yes	Yes	No	Yes	Yes	Yes	Yes	No	No	Moderate
Zielinski et al. 2004[74]	CA	CA	No*	Yes	Yes	Yes	No	No	Yes	No	No	Low
Medical Service Advisory Committee (MSAC) 2004[76]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	High
Pai et al. 2003[57]	CA	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	High
Medical Service Advisory Committee (MSAC) 2003[77]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	High

Checkpoint	Was an 'a priori' design provided?	Was there duplicate study selection and abstraction?	Was a comprehensive literature search performed?	Was the status of publication (i.e., English only) used as inclusion criterion?	Was a list of studies (included and excluded) provided?	Were the characteristics of the included studies assessed and documented?	Was the scientific quality of the included studies assessed and documented?	Was the scientific quality of the included studies used appropriately in formulating the conclusions?	Were methods used to combine the findings of studies appropriate?	Was the likelihood of publication bias assessed?	Was any conflict of interest stated?	Overall Rating
Sarmiento et al. 2003[67]	CA	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No	High
Tsao et al. 2001[78]	CA	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	No	Moderate
Dumler 2001[68]	No	CA	No	CA	No	No	No	No	Yes	No	No	Low

Source: Shea et al. 2007, AMSTAR: a measurement tool to assess the quality of systematic reviews.[52]

ECRI Institute applied overall assessment ratings using the following criteria: "High" if a study had mostly yes's (at least 8), "Moderate" if a mix of yes, no's, and can't answer, and "Low" if a study had mostly no's (at least 8)

CA Can't answer
NA Not applicable

* The authors of the review only reported searching MEDLINE.

Table 30. Systematic Reviews of Test Properties of Molecular Tests for Infectious Diseases

Review	Purpose	Quality of Review	Outcome	Number of Included Studies	Type of Molecular Test	Reference Standard	Reported Results	Authors' Conclusions
National Institute for Health Research, 2007[69]	To evaluate the accuracy of the following tests in patients with suspected TB: NATs tests, amplification molecular probes tests, serodiagnostic and biochemical assays, and phage-based tests[1]	High	Diagnostic accuracy	207 (106 used commercial tests and 101 used in-house tests)	PCR	Standard tests (mostly culture)	NAT accuracy was far superior when applied to respiratory samples as opposed to other body fluids. The specificity of NATs was high when applied to body fluids, for example TB meningitis and pleural TB, but sensitivity was poor, indicating that these tests cannot be used reliably to rule out TB.	The NATs provide a reliable way of increasing the specificity of diagnosis, but sensitivity is too poor to rule out disease, especially in smear-negative (paucibacillary) disease where clinical diagnosis is equivocal and where the clinical need is greatest. For extra-pulmonary TB, NATs have high specificity and could be used alongside adenosine deaminase (ADA).
Greco et al. 2006[64]	To analyze the accuracy of commercially-based NATs used for the diagnosis of TB in smear positive and negative respiratory samples	High	Diagnostic accuracy	63	PCR, SDA, TMA, LCR	Culture	Pooled sensitivity and specificity were 96% and 85% among smear positive samples, and 66% and 98% among smear negative samples. Test type had no effect on the DOR, but seemed to be correlated with sensitivity and specificity, probably via threshold effect.	Commercial NATs can be confidently used to exclude TB in patients with smear positive samples, and to confirm TB in a proportion of smear negative cases. The methodological characteristics of primary studies have a considerable effect on reported diagnostic accuracy.

Review	Purpose	Quality of Review	Outcome	Number of Included Studies	Type of Molecular Test	Reference Standard	Reported Results	Authors' Conclusions
Morgan et al. 2005[65]	To evaluate the overall accuracy of line probe assay in the detection of rifampicin (RIF)-resistant TB	High	Diagnostic accuracy	15	INNO-LIPA Rif. TB kit (Inn genetics, Belgium)	Culture	Twelve of the 14 studies that applied LIPA to isolates had sensitivity greater than 95%, and 12 of 14 had specificity of 100%. The four studies that applied LIPA directly to clinical specimens had 100% specificity, and sensitivity ranged between 85% and 100%.	LIPA is a highly sensitive and specific test for the detection of rifampicin resistance in culture isolates. The test appears to have relatively lower sensitivity when used directly on clinical specimens.
Flores et al. 2005[66]	To determine factors associated with heterogeneity and higher diagnostic accuracy in studies that evaluated in-house PCR for the diagnosis of TB.	High	Diagnostic accuracy	84	PCR	Culture	The sensitivity and specificity estimates varied widely: sensitivity varied from 9.4% to 100%, and specificity estimates ranged from 5.6% to 100%. In meta-regression analysis, the use of IS6110 as a target, and the use of nested PCR methods appeared to be significantly associated with higher diagnostic accuracy.	Estimates of accuracy of in-house NATs for TB are highly heterogeneous, rendering any estimate of the clinical utility difficult.
Pai et al. 2004[56]	To determine the overall accuracy of NATs used in the diagnosis of tuberculous pleuritis and to identify factors associated with heterogeneity of between-study results.	High	Diagnostic accuracy	40 (26 used in-house tests and 14 commercial tests)	PCR	Culture	Commercial tests had a low overall sensitivity (62%) and high specificity (98%). With the in-house tests, both sensitivity and specificity estimates were significantly heterogeneous.	Commercial NATs may have a potential role in confirming TB. However, these tests have low and variable sensitivity, and may not be useful excluding disease. The accuracy of in-house tests is poorly defined because of heterogeneity in study results.

Review	Purpose	Quality of Review	Outcome	Number of Included Studies	Type of Molecular Test	Reference Standard	Reported Results	Authors' Conclusions
Pai et al. 2003[57]	To determine the accuracy of NATs for tuberculous meningitis	High	Diagnostic accuracy	49 (35 used in-house tests and 14 commercial tests)	PCR	Culture	The summary estimates in 14 studies with commercial NAA tests were: sensitivity 56% (95% CI: 46% to 66%), specificity 98% (97% to 99%), positive likelihood ratio 35.1 (19.0 to 64.6), negative likelihood ratio 0.44 (0.33 to 0.60), and diagnostic odds ratio 96.4 (42.8 to 217.3). In the 35 studies with in-house tests, the summary accuracy could not be established with confidence because of wide variability in test accuracy.	Commercial kits show a potential role in confirming tuberculous meningitis diagnosis. However, their overall low sensitivity precludes the use of these tests to rule out tuberculous meningitis with certainty.
Sarmiento et al. 2003[67]	To summarize diagnostic accuracy of PCR methods for the diagnosis of smear-negative TB, identify factors that account for differences in diagnostic accuracy of PCR, and describe characteristics that should be emphasized in future studies	High	Diagnostic accuracy	50	PCR	Culture	Sensitivity and specificity of PCR ranged from 9.0% to 100% and from 25 to 100%, respectively.	PCR is not consistently accurate enough to be routinely recommended for the diagnosis of smear-negative TB. Future studies of PCR should be conducted by patient and type of respiratory specimen, blindly, by using a reference standard that combines culture and clinical criteria.

Review	Purpose	Quality of Review	Outcome	Number of Included Studies	Type of Molecular Test	Reference Standard	Reported Results	Authors' Conclusions
Dumler 2001[68]	To inform laboratory staff and practitioners about the applications and limitations of molecular microbiological methods for diagnosis of Lyme disease.	Low	Diagnostic accuracy	36	PCR	Culture	Median sensitivity ranged from 24% to 76% depending on specimen. Higher sensitivity levels were found in studies using either skin or urine specimens, lowest was in studies using cerebrospinal fluid. The overall median specificity ranged from 99% to 100%.	Molecular assays for Lyme disease are best used with other diagnostic methods, and only in situations in which the clinical probability of Lyme disease is high.

[1] The results reported in the table are specific to NATs and other molecular tests.

DOR Diagnostic odds ratio
LCR Ligase chain reaction
LIPA Line probe assay
NATs Nucleic acid amplification tests
NR Not reported
PCR Polymerase chain reaction
SDA Strand displacement amplification
TB Pulmonary tuberculosis
TMA Transcription medical amplification

Table 31. Systematic Reviews of Tests Properties of Molecular Tests for Cancers

Review	Purpose	Quality of Review	Outcome	Number of Included Studies	Type of Molecular Test	Reference Standard	Reported Results	Authors Conclusions
Blue Cross Blue Shield, 2008[80]	To examine whether the use of gene expression profiling improves outcomes when used to decide whether risk of recurrence is low enough to do without adjuvant chemotherapy for early stage breast cancer.	Moderate	Breast cancer recurrence	Four studies on Onco*type*DX met inclusion criteria	Onco*type*DX, MammaPrint, and Breast Gene Expression Ratio	Conven-tional risk assess-ment tools	Insufficient evidence to determine if MammaPrint and the Breast Gene Expression Ratio is better than conventional risk assessment tools in predicting recurrence. Women classified as high risk by conventional methods and reclassified as low risk by Onco*type*DX have a recurrence rate of 10% to 14%.	The authors concluded that "Onco*type*DX provides information about the risk of recurrence that is incremental to conventional classifiers used to predict risk."

Review	Purpose	Quality of Review	Outcome	Number of Included Studies	Type of Molecular Test	Reference Standard	Reported Results	Authors Conclusions
Seidenfield et al. 2008[79]	A systematic review of the evidence on using *HER2* testing to manage cancer patients in terms of potential response to trastuzumab among breast cancer patients who have negative, equivocal, or discordant *HER2* assay results, guide to selection of breast cancer treatments other than trastuzumab (e.g., chemotherapy), the use of serum *HER2* to monitor treatment response or disease progression in breast cancer, and the use of *HER2* testing to manage patients with ovarian, lung, prostate, or head or neck tumors.	High	Time to event, tumor response, quality of life	3 articles plus 1 abstract on use of trastuzumab among *HER2*-negative or discordant breast cancer patients; 26 articles on chemotherapy or hormonal therapy for breast cancer; 15 articles on plasma or serum *HER2* in patients treated for breast cancer; and 26 articles on serum or tissue *HER2* in patients with lung cancer, ovarian cancer, head and neck cancer, and prostate cancer.	PathVysion HER2 DNA Probe Kit (fluorescence in situ hybridization, FISH), INFORM HER2/neu Probe (FISH), *HER2* FISH pharmDx Kit, Spot-Light (CISH), EnzMet GenePro (SISH), Hercep Test (immunohistochemistry, IHC), PATHWAY (IHC)	NR	The evidence is weak on outcomes of trastuzumab added to chemotherapy for *HER2*-equivocal, discordant, or negative patients and when comparing chemotherapy outcomes in *HER2* positive and negative patient subgroups. Evidence is also weak regarding differences by *HER2* status for outcomes of chemotherapy for advanced or metastatic disease and for testing malignancies of lung, ovary, head and neck, or prostate.	Overall, the authors concluded that few studies directly investigated the questions of interest and that future cancer therapy trial protocols should incorporate elements to facilitate robust analyses of the use of *HER2* status and other biomarkers for managing treatment.

Review	Purpose	Quality of Review	Outcome	Number of Included Studies	Type of Molecular Test	Reference Standard	Reported Results	Authors Conclusions
Marchionni et al. 2008[38]	To evaluate the evidence about the use of gene expression profiling in breast cancer	High	Diagnostic accuracy	21	Onco*type* Dx (uses RT-PCR); MammaPrint (uses microarray technology); and Breast Cancer Profiling (BCP, uses RT-PCR)	Standard prognostic approaches (e.g., tumor type, *HER-2* status)	Based on their review of the evidence, the authors determined that the Onco*type* DX® assay had the strongest evidence for its ability to improve assessment of prognosis than standard risk stratification, at least in ER positive, lymph node negative, tamoxifen-treated women considering adjuvant chemotherapy.	The authors point out that there is still uncertainty about how best to incorporate the results of genetic profiling into clinical decision-making and how best to use the conventional predictors to which results are compared.

Review	Purpose	Quality of Review	Outcome	Number of Included Studies	Type of Molecular Test	Reference Standard	Reported Results	Authors Conclusions
Mocellin et al. 2007[71]	To evaluate published studies on PCR-based detection of melanoma cells in sentinel lymph nodes (SLN) for the purpose of assessing the prognostic value.	Moderate	TMN stage, disease recurrence, and/or survival (either overall [OS] or disease free [DFS])	22	PCR	HE staining combined with IHC	PCR status correlated with both TNM stage and disease recurrence. PCR positivity was also associated with worse overall (hazard ratio, 5.08, p = 0.002) and disease-free survival (hazard ratio, 3.41, p <0.001)	The available evidence is somewhat conflicting and probably is not sufficient to conclude that PCR status is a prognostic indicator reliable enough to be implemented clinically in the therapeutic decision-making process.

Review	Purpose	Quality of Review	Outcome	Number of Included Studies	Type of Molecular Test	Reference Standard	Reported Results	Authors Conclusions
Dendukuri et al. 2007[72]	To evaluate the concordance and cost-effectiveness of using various test strategies involving immunohisto-chemistry (IHC) and fluorescence in situ hybridization (FISH) to test *HER2* status.	High	Diagnostic accuracy of FISH when IHC is positive and cost-effective-ness	18	FISH and IHC	NR	Confirmation of the *HER2* status by FISH in cases that received a score of 3+ reduced the percentage of false-positive to 0% and increased the percentage of accurately determined *HER2* results to 97.6%. The test strategy of performing FISH testing in all cases of breast cancer was associated with a median incremental cost-effectiveness ratio of $8,401 per case.	The testing strategy with the lowest cost-effectiveness ratio involved screening all newly diagnosed cases of breast cancer with IHC and confirming scores of 2+ and 3+ with FISH.

Review	Purpose	Quality of Review	Outcome	Number of Included Studies	Type of Molecular Test	Reference Standard	Reported Results	Authors Conclusions
Lyman et al. 2006[43]	To systematically review the test performance of various classes of gene expression signatures in women with early stage breast cancer (ESBC)	Moderate	Distance recurrence-free survival	17	Microarray assays	NR	The overall sensitivity for distant recurrence-free survival was 80.6%, specificity was 53.6%, positive likelihood ratio was 1.78, negative likelihood ratio was 0.38, diagnostic odds ratio was 5.53%, positive predictive value was 37.7, and negative predictive value was 92%.	Gene expression profiles based on microarray analysis show early promise for predicting survival in patients with breast cancer. However, the use of these assays in therapeutic decision-making must consider the limitations of assay test performance and the specific patient population being tested.

Review	Purpose	Quality of Review	Outcome	Number of Included Studies	Type of Molecular Test	Reference Standard	Reported Results	Authors Conclusions
Pakos et al. 2004[73]	To evaluate the relation of *TP53* status with response to chemotherapy and/or clinical outcome in osteosarcoma	Moderate	Response to chemo-therapy (a cut off of 90% necrosis was used to separate responders from non-responders) and mortality	16	9 studies used IHC, 4 PCR, and 3 PCR+IHC	NR	*TP53* status had no discriminating ability to identify poor versus good responders to chemotherapy. Sensitivity and specificity of all studies was 50% and 56%, respectively. Separate analyses with studies using IHC or PCR were similar. In studies using RT-PCR there was a statistically significant association between *TP53* alterations and worse 2-year survival.	*TP53* status is not associated with the histologic response to chemotherapy in patients with osteosarcoma, whereas *TP53* gene alterations may be associated with decreased survival.

Review	Purpose	Quality of Review	Outcome	Number of Included Studies	Type of Molecular Test	Reference Standard	Reported Results	Authors Conclusions
Paraskevaidis et al. 2004[75]	To systematically review and critically appraise the current evidence of the reliability of HPV DNA testing during the post-treatment surveillance of cervical intraepithelial neoplasia (CIN).	Moderate	Recurrent or residual disease	11	PCR or HC II	Hysterecto my and pathologic-al examin-ation, cytology and/or colposcopy, biopsy, or combined methods.	The sensitivity of HPV DNA testing detecting treatment failures reached 100% in four studies and reached a modest performance, ranging from 47% to 67% in two studies. The specificity of the test differed across studies, ranging from 44% to 95%.	A positive HPV test, even in the presence of normal cytology, may pick up a treatment failure early and accurately. However, cytology and colposcopy may still be needed in order to rule out false positive and false negatives.

Review	Purpose	Quality of Review	Outcome	Number of Included Studies	Type of Molecular Test	Reference Standard	Reported Results	Authors Conclusions
Zielinski, G. 2004[74]	To evaluate the utility of hrHPV testing in monitoring women after treatment for cervical intraepithelial neoplasia grade 3 (CIN-3)	Low	Recurrent or residual disease	20	PCR or HC I or II	hrHPV test compared to margin resection or cervical cytology	The negative predictive value for recurrent/ residual disease of hrHPV testing was 98%, of resection margins 91%, and that of cervical cytology 93%. When hrHPV testing was performed in conjunction with cytology, the sensitivity was 96%, specificity was 81%, the associated positive predictive value was 46%, and the negative predictive value was 99%.	The authors propose to include hrHPV testing in conjunction with cytology for monitoring women treated for CIN 3. Some follow-up visits for women testing negative with both hrHPV and cytology can be skipped. In Western countries, this could mean that for women, double negative at 6-months, retesting at 12-months should be skipped while keeping the 24-month follow-up visit.

Review	Purpose	Quality of Review	Outcome	Number of Included Studies	Type of Molecular Test	Reference Standard	Reported Results	Authors Conclusions
Medical Service Advisory Committee (MSAC) 2004[76]	To evaluate the evidence to answer the following questions: Do targeted RT-PCR assays increase the proportion of patients who are recognized to have *BCR-ABL* positive acute lymphocytic leukemia (ALL), which defines a specific therapeutic strategy?; Does repeated qualitative or quantitative PCR testing post-treatment influence management and prediction of relapse?	High	Diagnostic accuracy	33	PCR	For the assess-ment of validity of PCR for the detection of *BCR-ABL-ALL* at diagnosis was a combina--tion of cytogenetic testing, FISH testing, and prognosis. For monitoring the reference was relapse.	**Diagnostic accuracy in diagnosis**: Based on pooled analysis of 27 studies, PCR was estimated to have a sensitivity of 94.8% (95%CI: 92.6 to 96.5) and specificity of 97.3% (95%CI: 92.4 to 99.4). **Diagnostic accuracy in monitoring**: Based on pooled analysis of 6 studies, the pooled diagnostic odds ratio was 4.7 (95%CI: 2.2 to 10.3).	The use of PCR, with improved sensitivity compared with cytogenetic testing, could be expected to produce improved patient outcomes. PCR may also predict hematological relapse early, although with imperfect sensitivity and specificity compared with other diagnostic modalities.

Review	Purpose	Quality of Review	Outcome	Number of Included Studies	Type of Molecular Test	Reference Standard	Reported Results	Authors Conclusions
Medical Service Advisory Committee (MSAC) 2003[77]	To evaluate the evidence on the use of PCR in the diagnosis of patients with *BCR-ABL* gene rearrangement in chronic myeloid leukemia.	High	Diagnostic accuracy	55	PCR	Not clearly reported in all included studies (cyto-genetic testing in most studies).	Based on pooled analysis of 17 studies, PCR was 100% (95%CI: 99 to 100) sensitive in patients *BCR-ABL* positive and 79% (95%CI: 49 to 95) sensitive in patients *BCR-ABL* negative. Combined PCR and cytogenetic testing was 99% (95%CI: 98 to 100) sensitive at diagnosis.	The use of PCR testing in both diagnosis and monitoring of CML provided both clinicians and patients with earlier and/or more accurate information, which was of benefit.

Review	Purpose	Quality of Review	Outcome	Number of Included Studies	Type of Molecular Test	Reference Standard	Reported Results	Authors Conclusions
Tsao et al. 2001[78]	To systematically review the use of RT-PCR for tyrosinase messenger RNA as a molecular serum marker for metastatic melanoma.	Moderate	Diagnostic accuracy	23	RT-PCR	History, physical examination, chest radiography, and complete blood cell count	Results of RT-PCR for tyrosine messenger RNA were positive in 18% of patients for stage I, 28% for stage II, and 45% for stage IV disease. Specificities were 100% in all but 1 study. Results of RT-PCR were positive in only 0.4% of healthy controls with nonmelanoma cancer.	The lack of data on the outcome of stage I, II, and II patients who were RT-PCR positive and the low prevalence of RT-PCR positivity in patients with known stage IV disease limits the applicability of RT-PCR as a prognostic indicator.

HC I or II Hybrid Capture I or II. This test is based on direct HPV DNA detection by hybridization of HPV target DNA with a cocktail of full-length HPV type specific RNAs, followed by capturing the hybrids to a solid phase. The HC II test contains more HPV types than the preceding HC I test.
IHC Immunohistochemistry
PCR Polymerase chain reaction
RT-PCR Reverse transcriptase-polymerase chain reaction

Table 32. Systematic Reviews of Test Properties of Molecular Tests for Predicting Drug Reactions

Review	Purpose	Quality of Review	Outcome	Number of Included Studies	Type of Molecular Test	Reference Standard	Reported Results	Authors' Conclusions
Blue Cross and Blue Shield Association 2008[81]	*CYP2D6* testing and consequent alteration of treatment regimen in *CYP2D6* poor metabolizers compared to no testing and no alteration of decision to treat with tamoxifen for patients with high risk for or with breast cancer.	Moderate	Disease prevention, improved time to recurrence, recurrence-free, and/or overall survival	4 prospective cohort studies addressed the association of genotype with plasma levels of active tamoxifen metabolite 7 retrospec-tive cohort studies addressed the association of genotype with clinical outcomes	Roche AmpliChip CYP450 Test	DNA sequencing (gold standard) or methods comparison (e.g., PCR)	Three prospective studies provided consistent evidence that *CYP2D6* nonfunctional variant alleles that determine patient's tamoxifen metabolism status (poor, intermediate, or high) are associated with significantly reduced plasma levels of endoxifen, the most bioavailable of tamoxifen active metabolites. However, endoxifen levels overlap across all genotypes, suggesting that *CYP2D6* genetic variability does not explain all variability in endoxifen levels. The relationship between endoxifen plasma concentration and clinical outcomes was not established by the evidence. There was no direct evidence of clinical utility (whether use of *CYP2D6* genotype testing for endocrine therapy regimen selection improves recurrence and survival outcomes.	Overall, the authors concluded that the evidence was insufficient to permit conclusions regarding the use of *CYP2D6* genotyping for directing endocrine therapy regimen selection for women at high risk for or with breast cancer.

Review	Purpose	Quality of Review	Outcome	Number of Included Studies	Type of Molecular Test	Reference Standard	Reported Results	Authors' Conclusions
Blue Cross and Blue Shield of Massachusetts 2008[206]	To determine whether a pharmacogenomics-based treatment regimen is superior to a standard regimen for the eradication of H. pylori, and whether the use of a pharmaco-genomics-based treatment regimen improves health outcomes compared to standard treatment.	High	Successful eradication of H. pylori (which has been proven to reduce the recurrence of gastritis and peptic ulcer disease.	1 randomized trial	Roche AmpliChip CYP450 Test	DNA sequencing (gold standard) or methods comparison (e.g., PCR)	The single study that met the inclusion criteria for this report compared a pharmacogenomics-based treatment regimen for H. pylori with a standard treatment regimen. Genetic testing was performed on H. pylori isolates to determine sensitivity to clarithromycin. Treatment for the pharmacogenomics group was determined based on sensitivity status (extensive metabolizer to poor metabolizer). Overall, the results of the study indicated that H. pylori eradication rates at 1 month were 96% in the pharmacogenomics-based treatment group versus 70% in the standard care group (a statistically significant difference, $p < 0.001$). Eradication rates were greater for extensive metabolizers than poor metabolizers.	Because only one study met the inclusion criteria for this review, the authors concluded that the evidence did not permit conclusions on whether the use of a pharma-cogenomics-based treatment regimen for H. pylori improves eradication rates or net health outcomes.

Review	Purpose	Quality of Review	Outcome	Number of Included Studies	Type of Molecular Test	Reference Standard	Reported Results	Authors' Conclusions
Matcher et. al. 2006[37]	To determine if testing for *CYP450* polymorphisms in adults entering SSRI treatment for non-psychotic depression leads to improvement in outcomes, or if testing results are useful in medical, personal, or public-health decision-making.	High	Analytic validity (sensitivity and specificity), drug metabolism, predicting SSRI efficacy, improved patient outcomes, adverse events, management decision-making.	37	Most studies used Roche Amplichip ® CYP450 Test	DNA sequencing (gold standard) or methods comparison (e.g., PCR)	Three of the five studies on drug metabolism showed that *CYP2C19* PMs have significantly higher area under the curve (AUC, which is an assessment of bioavailability), longer half-life, and reduced oral clearance of the parent drug, and lower maximum plasma concentration of the metabolite of each drug. Similar findings were found in a study of *CYP2D6* in healthy volunteers after a single dose of paroxetine, while another study of *CYP2D6* using multiple doses of paroxetine found no significant difference between PMs and EMs. The remaining 11 studies showed mixed results because of heterogeneity and small sample sizes. The findings from studies on *CYP450* testing and efficacy of SSRIs were mixed with most finding either no difference in the proportion of responders among *CYP2D6* EMs, IMs, and PMs, or that plasma blood levels did not predict treatment response. Of the nine studies that addressed *CYP450* testing and adverse drug reactions, three reported adverse effects in CYP PMs only as a secondary finding. Of the remaining six studies, three reported no difference in adverse effects between *CYP2D*^ PMs and EMs, while a fourth study reported no difference in adverse effects between the combined PM+IM and EM+UM groups. The remaining two studies found a greater prevalence of gastrointestinal adverse effects in PMs compared to EMs.	A paucity of good-quality data addressing the questions of whether testing for *CYP450* polymorphism in adults entering SSRI treatment for non-psychotic depression leads to improvement in outcomes, or whether testing results are useful in medical, personal, or public health decision-making.

Review	Purpose	Quality of Review	Outcome	Number of Included Studies	Type of Molecular Test	Reference Standard	Reported Results	Authors' Conclusions
Morgan et al. 2005[65]	To evaluate the overall accuracy of line probe assay in the detection of rifampicin (RIF)-resistant TB	High	Diagnostic accuracy	15	INNO-LIPA Rif. TB kit (Inn genetics, Belgium)	Culture	Twelve of the 14 studies that applied LIPA to isolates had sensitivity greater than 95%, and 12 of 14 had specificity of 100%. The four studies that applied LIPA directly to clinical specimens had 100% specificity, and sensitivity ranged between 85% and 100%.	LIPA is a highly sensitive and specific test for the detection of rifampicin resistance in culture isolates. The test appears to have relatively lower sensitivity when used directly on clinical specimens.

Review	Purpose	Quality of Review	Outcome	Number of Included Studies	Type of Molecular Test	Reference Standard	Reported Results	Authors' Conclusions
Sanderson et al. 2005[83]	To examine the strength and quality of existing evidence about *CYP2C9* gene variants and clinical outcomes in warfarin-treated patients.	Moderate	Drug dose, bleeding events	11	PCR	NR	Mean difference in daily warfarin dose: for *CYP2C9*2*, the reduction was 0.85 mg (0.60 to 1.11 mg), a 17% reduction. For *CYP2C9*3*, the reduction was 1.92 mg (1.37 to 2.47 mg, a 37% reduction. For *CYP2C9*2* or **3*, the reduction was 1.47 mg (1.24 to 1.71 mg), a 27% reduction. The relative bleeding risk for *CYP2C9*2* was 1.91 (1.16 to 3.17) and for *CYP2C9*3*, 1.77 (1.07 to 2.91). For either variant, the relative risk was 2.26 (1.36 to 3.75).	Patients with *CYP2C9*2* and *CYP2C9*3* alleles have lower mean daily warfarin doses and a greater risk of bleeding. Testing for gene variants could potentially alter clinical management in patients commencing warfarin. Evidence for the clinical utility and cost-effectiveness of genotyping is needed before routine testing can be recommended.

NR Not reported
PCR Polymerase chain reaction

Table 33. Published Studies of Proficiency Testing for FISH Assays

Study	Design	Organization	Reference Materials	Number of Participating Laboratories	Number of Laboratories Returning Results	Results	Factors that Contributed to Variability
Bartlett et al. 2007[111]	Present results of a pilot proficiency testing survey for *HER-2/neu*.	National External Quality Assessment Scheme (NEQUAS), United Kingdom (UK)	Five breast cancer cell lines, two with amplification and three without. Laboratories tested each line three times. Samples were distributed fixed on slides.	6 reference centers and 31 laboratories	The laboratories returned 78 sets of data (out of 93 possible sets) and the reference centers returned 29 complete sets of results.	60% of all results were "acceptable," however 22.4% of results returned were "inappropriate," including 13 cases (4.2%) where a misdiagnosis would have been made had these been clinical specimens.	Most of the incorrect diagnoses were from one cell line that has low-level *HER2* amplification.
Persons et al. 2006[112]	Present the results of proficiency testing surveys from 2000 to 2004 for *HER-2/Neu*; years 2000 and 2001 were previously reported in 2002, and are thus not tabled here	College of American Pathologists (CAP)	Nine breast cancer cases with known *HER-2/neu* status, 4 with high levels of amplification, 4 without amplification, and a case with low-level amplification. Samples were distributed as fixed paraffin-embedded tissue blocks.	2002 survey 90	90 (100%) returned results for the *HER-2/neu* positive specimen and 89 (99%) returned results for the low-level sample	All results for the high-level amplification sample were correct, but only 56% of laboratories correctly identified the low-level sample as amplified	All of the incorrect diagnoses were from one sample with low-level *HER2* amplification.
				2003 survey 127	127 (100%) returned results for the *HER-2/neu* negative specimen, and 124 (98%) returned results for the *HER-2/neu* positive specimen	97% correctly identified the negative specimen as negative; 76% correctly identified the low-level specimen as positive	Most of the variability was from one sample with low-level *HER2* amplification.
				2004 survey 139	139 (100%) returned results for the *HER-2/neu* positive specimen and 136 (98%) returned results for the *HER-2/neu* negative specimen.	94% correctly identified the positive specimen as positive, and 97% correctly identified the negative specimen as negative	Little variability was observed.

Study	Design	Organization	Reference Materials	Number of Participating Laboratories	Number of Laboratories Returning Results	Results	Factors that Contributed to Variability
Cell Markers and Cytogenetic Committees, CAP 2002[113]	Present the results of proficiency testing surveys from 2000 and 2001 for *HER-2/neu*	College of American Pathologists (CAP)	Two breast cancer cases with known *HER-2/neu* status one with amplification and one without amplification. Samples were distributed fixed on slides.	2000 survey 35	35 (100%)	All returned results were correct.	No variability was reported.
				2001 survey 63	49 (78%) returned results for the *HER-2/neu* negative specimen and 58 (92%) returned results for the *HER-2/neu* positive specimen.	All returned results were correct.	No variability was reported.
Mascarello et al. 2002[114]	Present the results of FISH proficiency testing surveys from 1997 to 2000.	College of American Pathologists (CAP) and American College of Medical Genetics (CAP-ACMG)	1997 survey Microdeletion of chromosome 22 in blood and a normal sample, both fixed to slides	131	116 (88.5%)	93.1% correctly identified the deletion and 85.1% correctly identified the normal sample	All laboratories used commercially available probes. There was no correlation between a laboratory's experience with a particular test and the laboratory's proficiency test performance. Laboratories with all correct interpretations on the proficiency tests were more likely to have written quality control plans.
			1998 survey 1 *BCR-ABL* gene fusion in blood fixed to slides with varying proportions of cells carrying the fusion	134	117 (87.3%)	97.4% correctly identified the fusion in a sample that was 98% neoplastic and 93.2% correctly identified the fusion in a sample that was 28% neoplastic	
			1998 survey 2 Microdeletion of chromosome 7 in blood and a normal sample fixed to slides	121	109 (90.1%)	97.2% correctly identified the deletion and 94.5% correctly identified the normal sample	
			1999 survey 1 *PML-RARA* gene fusion in blood and a normal sample fixed to slides	136	108 (79.4%)	99.1% correctly identified the fusion in a sample that was 92% neoplastic and 98.1% correctly identified the normal sample	

Study	Design	Organization	Reference Materials	Number of Participating Laboratories	Number of Laboratories Returning Results	Results	Factors that Contributed to Variability
			1999 survey 2 Prenatal detection of aneuploidy in cultured amniocytes fixed to slides	124	107 (86.3%)	100% correctly identified the trisomy 21 sample and 97.2% correctly identified the triploidy sample	
			2000 survey 1 *TEL-AML1* gene fusion in blood and a normal sample fixed to slides	113	103 (91.2%)	100% correctly identified the fusion in a sample that was 50% neoplastic, 96.1% correctly identified the fusion in a sample that was 20% neoplastic, and 99.0% correctly identified the normal sample	
			2000 survey 2 A microdeletion and a duplication of chromosome 15 in blood fixed to slides	151	146 (96.7%)	100% correctly identified the deletion, but only 4.1% correctly identified the duplication	

Table 34. Published Proficiency Studies of PCR-based Tests: PCR, General Aspects

Study	Design	Test(s)	Organization	Reference Materials	Number of Participating Laboratories	Number of Laboratories Returning Results	Results	Factors that Contributed to Variability
Orlando et al. 2007[115]	Present the results of a proficiency testing survey.	DNA extraction and PCR amplification	European Union Quality Control Concerted Action (EQUAL)	Blood samples as well as purified DNA.	213	175 (82%)	27% of laboratories had poor-quality DNA preparation. 3% of laboratories had a consistently low rate of amplification, and 10% did not identify the expected number of bands of amplified targets.	The reagents and PCR methods used by each laboratory were speculated to be the cause of the variability.
Ramsden et al. 2006[116]	Present the results of a proficiency testing survey.	Real-time quantitative PCR using Taqman probes, including extraction of RNA and preparation of cDNA.	European Union Quality Control Concerted Action (EQUAL)	Cell lines suspended in RNAlater. Reference standards and primers were provided. The target gene was *ABL*.	137	130 (94.9%)	Data from ten laboratories were excluded (results suggested the assays were contaminated or the data reported were incomplete). 20% of the remaining laboratories reported results that were lacking in precision and/or accuracy.	No differences in performance were observed for the >10 different PCR platforms used by the study participants.

Study	Design	Test(s)	Organization	Reference Materials	Number of Participating Laboratories	Number of Laboratories Returning Results	Results	Factors that Contributed to Variability
Raggi et al. 2005[117]	Present results of a proficiency testing survey	real-time PCR using Taqman probes	Italian Network for Quality Assurance of Tumor Biomarkers (INQAT)	cDNA prepared from three pools of RNA extracted from human cancers. Laboratories were provided with the same PCR probes and with a standard sample for reference curve preparation. The target was levels of human telomerase expression.	48	42 (87.5%)	Two laboratories reported results suggesting contamination of samples. Of the remaining 40 laboratories, only 12 correctly assayed all three samples. 17 reported imprecise results for at least one sample, 12 laboratories were unable to amplify anything from the third sample, 3 laboratories reported incorrect results for all three samples, and six laboratories were unable to correctly amplify the provided standards.	One particular PCR platform seemed to be over-represented in the group of laboratories that returned very poor results.
Birch et al. 2004[118]	Present results of a proficiency testing survey	DNA extraction, amplification by PCR, and analysis of products by electrophoresis.	Organized by the participating laboratories	Killed bacteria diluted into saline.	15	15 (100%)	Overall laboratories performed the analysis successfully.	Experience with bacteria did not affect the accuracy of the results. The laboratory itself was the most important factor, with some laboratories performing well on most of the tests and others not.

Study	Design	Test(s)	Organization	Reference Materials	Number of Participating Laboratories	Number of Laboratories Returning Results	Results	Factors that Contributed to Variability
Raggi et al. 2003[119]	Present results of a proficiency testing survey	DNA extraction, amplification by PCR, and analysis of products by electrophoresis.	Italian Network for Quality Assurance of Tumor Biomarkers (INQAT)	Pooled blood samples collected from healthy volunteers. Three samples and a reference standard were supplied to each laboratory. Laboratories were provided with probes to amplify three target DNA sequences, and amplification parameters for the probes were provided.	39	39 (100%)	Only 4 laboratories were rated "excellent". 13 were rated "good", 15 were "sufficient", 3 were "poor", and 4 were "unacceptable".	Poor quality DNA extraction contributed significantly to the variability. Efficiency of PCR amplification varied dramatically across laboratories as well.

Study	Design	Test(s)	Organization	Reference Materials	Number of Participating Laboratories	Number of Laboratories Returning Results	Results	Factors that Contributed to Variability
Braun et al. 1998[120]	Present the results of a pilot proficiency testing survey	DNA preparation from whole blood, PCR amplification, and agarose gel electrophoresis of PCR products.	Reference Institute of Bioanalysis of the German Society of Clinical Chemistry	Blood from a volunteer and DNA prepared from the blood of a volunteer. PCR primers directed to two different targets were provided.	50	45 (90.0%)	Most of the participants obtained comparable results. The mean quality of DNA prepared using commercial kits was much lower than the DNA prepared using laboratory-developed methods. The majority of participants over-estimated the exact size of the PCR products.	Nonoptimal PCR reagents and parameters were speculated to be the major source of variability.

Table 34a. Published Proficiency Studies of PCR-based Tests: PCR-based Detection and Typing of Viruses

Study	Design	Test(s)	Organization	Reference Materials	Number of Participating Laboratories	Number of Laboratories Returning Results	Results	Factors that Contributed to Variability
Stelzer-Braid et al. 2008[136]	Present the results of a proficiency testing program.	PCR tests to detect avian influenza viruses.	Consortium of academics	Three panels of viruses grown in culture, inactivated, and diluted in saline.	29	29 (100%)	80 to 88% of the laboratories returned correct results. Very dilute samples were often reported as false-negatives.	Samples with low viral titers produced the greatest variability in results. The sensitivity of the tests did, however, improve slightly between panels 2 and 3.
Wang et al. 2008[135]	Present the results of five years of a proficiency testing program.	PCR tests to detect hepatitis C virus.	National Center for Clinical Laboratories (China)	Ten different panels of plasmids carrying viral DNA diluted in plasma and freeze-dried.	153 to 233, depending on which panel.	Not reported	Overall, 98.6% of negative samples and 96.7% of positive samples were correctly identified. The most concentrated samples had a 99.4% detection rate, and the most dilute samples had an 87.7% detection rate. In 2003, only 30.5% of the laboratories correctly identified the amount of virus in the samples, compared to 2007 when 91.2% correctly identified the amount of virus in the sample.	Samples with low viral titers produced the greatest variability in results. The improvement in results over time was attributed to the development of higher-quality commercial kits.

Study	Design	Test(s)	Organization	Reference Materials	Number of Participating Laboratories	Number of Laboratories Returning Results	Results	Factors that Contributed to Variability
Chalker et al. 2007[121]	Present the results of a proficiency testing program.	PCR tests to detect hepatitis C virus.	National External Quality Assessment Scheme (NEQAS)	Nine different panels of hepatitis virus diluted in plasma and freeze-dried.	52 to 159, depending on which panel	Not reported	The accurate detection of hepatitis C and its genotype varied from 86.9% to 100%.	Samples with low viral titers produced the greatest variability in results. False-positives of negative samples were very rare.
Niedrig et al. 2006[122]	Test whether labora-tories have improved since they participated in a previous proficiency testing survey.	PCR-based tests to identify and genotype orthopoxviruses (monkeypox, cowpox, vaccinia virus)	European Network for Diagnostics of Imported Viral Diseases (ENIVD)	Viral cultures irradiated to inactivate the viruses and diluted to various levels.	33	33 (100%)	Results were not significantly improved since the first survey. Laboratories had a greater than 80% detection rate above 56,234 copies per ml. 23 out of 27 laboratories were able to correctly genotype, and 9 were able to correctly quantitate the viruses. 22 false-positive results were returned, but 18 of these were from five laboratories.	Laboratories that used either real-time PCR or a commercial assay kit had more accurate results than laboratories that used conventional PCR or nested PCR. The use of commercial sample preparation kits did not influence the accuracy of the results.

Study	Design	Test(s)	Organization	Reference Materials	Number of Participating Laboratories	Number of Laboratories Returning Results	Results	Factors that Contributed to Variability
Templeton et al. 2006[123]	Present the results of a proficiency testing survey.	PCR tests to detect respiratory viruses.	Quality Control for Molecular Diagnostics (QCMD)	Stocks of viruses prepared at reference laboratories were diluted into media. Viruses included influenza, rhinovirus, adenovirus, and coronavirus.	17	17 (100%)	93.75% of high-titer samples were correctly identified; 76.5% of medium-titer and 47.0% of low-titer samples were correctly identified.	The overall performance was not dependent on the methodology chosen by the laboratory; high-quality results instead seem to be dependent on the laboratory's internal quality control and optimization of the assay used.
Drosten et al. 2004[124]	Present the results of a proficiency testing survey	PCR-based methods of detecting the presence of viruses that cause severe acute respiratory syndrome (SARS).	World Health Organization SARS Reference and Verification Network	Inactivated viral RNA material extracted from viral cultures and diluted into plasma.	58	58 (100%)	Of 58 participants, 51 correctly detected virus in all samples >9,400 RNA copies per milliliter and none in negative samples.	The 14 of the 58 laboratories that used commercial kits had significantly more accurate results.
Mancini et al. 2004[125]	Present the results of a proficiency testing survey.	PCR tests to detect hepatitis C.	Italian Society of Clinical Microbiology (AMCLI)	Dilutions of hepatitis C in plasma.	17	17 (100%)	15 of the 17 laboratories used commercial kits, and 2 used homebrew assays. Only 1.4% of results were incorrect.	Little variability was observed. Errors were generally made only on low-titer samples.
Schloss et al. 2003[126]	Present the results of a proficiency testing survey.	PCR-based detection of herpes simplex virus.	European Union Quality Control Concerted Action (EQUAL)	Panels of virus diluted to various degrees.	66 panel 1, 77 panel 2	76 data sets were reported for panel 1, 78 data sets were reported for panel 2	30% of data sets were correct for the entire panel 1; 28% of data sets were correct for the entire panel 2.	Lack of sensitivity of tests caused the most errors.

Study	Design	Test(s)	Organization	Reference Materials	Number of Participating Laboratories	Number of Laboratories Returning Results	Results	Factors that Contributed to Variability
Schirm et al. 2002[127]	Present results of a proficiency testing survey	PCR-based tests to detect hepatitis C virus	European Union Quality Control Concerted Action (EQUAL)	Hepatitis C virus diluted into plasma	Panel 1- 57 Panel 2- 81	Panel 1: 45 qualitative and 35 quantitative data sets Panel 2: 75 qualitative and 48 quantitative data sets	Panel 1: 1.3% false-positive rate. Adequate or better scores on qualitative results were achieved in 84% of samples. Panel 2: 0.8% false-positive rate. Adequate or better scores on qualitative results were achieved on 80% of samples.	Detection of low-titer samples was often inadequate.
Valentine-Thon et al. 2001[128]	Report and compare the results of two different proficiency panels	PCR detection and quantitation of hepatitis B virus	European Union Quality Control Concerted Action (EQUAL)	Hepatitis B virus diluted in human plasma. Each panel had 2 negative samples and 6 positive samples with a range of viral copies per ml.	Panel 1, 42 Panel 2, 51	Panel 1: 20 qualitative data sets and 37 quantitative data sets Panel 2: 25 qualitative data sets and 47 quantitative data sets	Panel 1: an adequate or better score was obtained on 77.2% of the data sets Panel 2: an adequate or better score was obtained on 68.1% of the data sets. Home-brew qualitative PCR assays performed better than commercial quantitative assays because many of the assays were not sensitive enough to detect clinically relevant but low viral copy levels.	False-positive rates were extremely low. Most variability came from varying sensitivity of assays to detect low viral copy levels.

Study	Design	Test(s)	Organization	Reference Materials	Number of Participating Laboratories	Number of Laboratories Returning Results	Results	Factors that Contributed to Variability
Van Vliet et al. 2001[129]	Present results of a proficiency testing survey.	PCR-based methods of detecting enteroviruses	European Union Quality Control Concerted Action (EQUAL)	Panel of 12 samples of various enteroviruses at various titers.	63	59 (93.7%) laboratories returned 71 data sets; one set was excluded from analysis.	66% of the 70 data sets were correctly interpreted.	Nested PCRs were associated with better results than other methods; but all methods achieved a maximum score at least once. Staff proficiency and laboratory facilities were speculated to account for much of the variability.
van Loon et al. 1999[130]	Present the results of a proficiency testing survey.	PCR-based methods of isolating, detecting and typing enteroviruses.	European Union Quality Control Concerted Action (EQUAL)	Cell lines infected with virus.	12	11 (91.7%) on isolating virus; 4 (33.3%) on PCR for detecting virus	Correct virus isolation results were obtained for 105 of 110 samples (95.5%, four false-negatives, one false-positive), and correct PCR results for 39 of 40 (97.5%, one false-negative).	Laboratories were more successful in isolating virus from monkey cells than from human cells. Major problems were seen with samples containing mixtures of enteroviruses and with enterovirus 71 or echovirus 4, with 9%, 50%, and 55% correct results, respectively.

Study	Design	Test(s)	Organization	Reference Materials	Number of Participating Laboratories	Number of Laboratories Returning Results	Results	Factors that Contributed to Variability
Read et al. 1998[131]	Present results of a specimen exchange/ quality control program	PCR detection of various viruses	Laboratory initiated by virology sections of Oxford and Cambridge PHL	Either original clinical specimens or cerebrospinal fluid spiked with diluted virus infected cell culture. Sixty specimens in nine batches were exchanged over six months.	2	2 (100%)	Discrepant results were obtained for only 2 of the specimens	Sensitivity differences due to different primer designs were observed. Transport of samples at ambient temperature was observed to reduce the sensitivity of tests to detect RNA.

Table 34b. Published Proficiency Studies of PCR-based Tests: PCR-based Methods of Detecting and Typing Bacteria

Study	Design	Test(s)	Organization	Reference Materials	Number of Participating Laboratories	Number of Laboratories Returning Results	Results	Factors that Contributed to Variability
Deplano et al. 2006[132]	Present results of a proficiency testing survey.	Genotyping of staphylococcus aureus	The reference laboratory for MRSA-staphocci, Brussels, Belgium	Panels of staphylo-coccus selected from the collection at the reference laboratory.	2 surveys were conducted, and 10 laboratories participated per survey	10 (100%)	100% of the laboratories correctly typed the samples, and all but one laboratory demonstrated 100% reproducibility.	No variability in the results was observed despite the wide range of methods used to perform the tests.
Muyldermans et al. 2005[133]	Present results of two proficiency testing surveys	Detection of B. pertussis	Belgian Centres for Molecular Diagnostics (CMD)	Panel 1 was a series of dilutions of three strains of B. pertussis collected in 2000; Panel 2 consisted of 5 species of Bordetella. Samples were prepared in saline and provided frozen on dry ice.	6 laboratories participated in the first survey and 9 participated in the second survey.	100% for both surveys	First panel: No false-positives were reported. Laboratories reported varying ability to detect low-copy-number samples. Second panel: There was one false-positive and varying ability to detect low-copy-number samples and varying ability to identify the other strains of Bordetella.	There was no apparent correlation between methods used and accuracy of results, except that choice of target sequence was critical for distinguishing B. pertussis from other Bordetella species.

Study	Design	Test(s)	Organization	Reference Materials	Number of Participating Laboratories	Number of Laboratories Returning Results	Results	Factors that Contributed to Variability
CTLTN 2004[134]	Present results of three years of a proficiency testing program.	Typing and detection of M. tuberculosis by RFLP and PCR	Canadian Tuberculosis Laboratories Technical Network (CTLTN)	Samples of different strains of M. tuberculosis prepared by the National Reference Center	2000: 5 RFLP, 5 for PCR 2001: 5 RFLP, 13 for PCR 2002: 3 RFLP, 12 for PCR	100% for all years	2000: consensus for RFLP typing ranged from 60 to 100%; 5 of 9 positive samples correctly identified, and 2 of 5 negative samples correctly identified by PCR 2001: 100% consensus for RFLP typing; 25 of 26 positive samples correctly identified and 12 of 13 negative samples correctly identified by PCR. 2002: 100% consensus for RFLP typing; 22 of 24 positive samples correctly identified; 12 of 12 negative samples correctly identified	There was little variability and laboratory proficiency improved over time.

Table 34c. Published Proficiency Studies of PCR-based Tests: PCR-based Methods of Detecting Genetic Alterations Related to Cancer

Study	Design	Test(s)	Organization	Reference Materials	Number of Participating Laboratories	Number of Laboratories Returning Results	Results	Factors that Contributed to Variability
Hsi et al. 2002[137]	Retro-spective review of data collected from a proficiency testing program over four years.	PCR methods of detecting bcl-2/JH translocation (t(14;18)(q32;q21))	College of American Pathologists (CAP)	24 specimens which included six samples containing the target translocation. Specimens were either cell lines or human tissue. Some specimens were frozen, and some were fixed and embedded in paraffin.	25 to 61 depending on which panel. Panels were sent out twice a year for four years.	Not reported.	819 major breakpoint region and 323 minor cluster region determinations were performed, with an overall correct response rate of 91% and 94%, respectively. No significant difference in correct response could be found for frozen versus paraffin-embedded tissues.	Despite great variability in methods and primer choice, there was little variability observed. Few laboratories used an internal control, and few laboratories reported knowledge of the sensitivity of their chosen assay method.
Bolufer et al. 2001[138]	Present the results of a proficiency testing survey	RT-PCR based methods of detecting *PML-RAR*	Spanish External Quality Assessment Program (EQAP)	RNA samples extracted from cell lines; cell lines; and plasmids.	18	Panel 1, 15 (83.3%); Panel 2, 15 (83.3%); Panel 3, 13 (72.2%); Panel 4, 8 (44.4%); Panel 5, 16 (88.9%).	Although the laboratories used diverse methods, most were able to correctly analyze samples with high levels of the target.	Many of the assays used were not sensitive enough to detect low levels of the target. A high level of contamination of reactions was observed.

Study	Design	Test(s)	Organization	Reference Materials	Number of Participating Laboratories	Number of Laboratories Returning Results	Results	Factors that Contributed to Variability
Bolufer et al. 1998[139]	Present results of a proficiency testing survey	RT-PCR detection of *PML-RAR alpha*	Spanish External Quality Assessment Program (EQAP)	cDNA samples prepared by the reference laboratory from bone marrow samples obtained from patients with acute promyelocytic leukemia (APL)	7 in the first round, 12 in the second round	A total of 69 data sets were returned out of 88 expected.	Amplification of the control gene was satisfactory in 90% of samples. There was an 83% concordance between laboratories for *PML/RARa* detection with similar results for the type of *PML/RRa* rearrangements. However, 17% disagreement still remained.	Lack of sensitivity of assays and inappropriate use of primers contributed to some of the errors. Most of the discrepant results occurred with a single sample out of the 9 samples provided.

Table 34d. Published Proficiency Studies of PCR-based Tests: DNA Sequencing

Study	Design	Test(s)	Organization	Reference Materials	Number of Participating Laboratories	Number of Laboratories Returning Results	Results	Factors that Contributed to Variability
Afshar et al. 2007[143] and Fry et al. 2007[207a]	Present results of a proficiency testing program.	DNA sequencing method of typing Legionella bacteria	European Working Group for Legionella Infections	Bacterial isolates grown in the laboratory.	16 first panel, 18 second panel, 29 third panel.	11 (69%) first panel; 18 (95%) second panel; 27 (93%) third panel	First panel, only 50% of results were correct; second panel, 56% were correct; third panel, 76% were correct.	Laboratories that routinely performed many sequencing assays per year performed significantly better.
Dorn-Beineke et al. 2006[140]	Report impact of a training program on laboratory perform-ance on a proficiency testing survey.	DNA sequencing	European Union Quality Control Concerted Action (EQUAL)	PCR products or plasmids. Primers were provided.	8	6 (75%)	The laboratories demonstrated a significant improvement in proficiency.	Not relevant
Ahmad-Nejad et al. 2006[141]	Present results of a proficiency testing survey.	DNA sequencing	European Union Quality Control Concerted Action (EQUAL)	Plasmid DNA, an amplified PCR product, and a finished sequencing reaction to be analyzed.	60	43 (71.7%)	There was considerable variation in both the quality of the sequencing data and in the interpretation of the data.	There was a correlation between quality of data and interpretation and the number of sequencing assays the laboratory performed each year.

Study	Design	Test(s)	Organization	Reference Materials	Number of Participating Laboratories	Number of Laboratories Returning Results	Results	Factors that Contributed to Variability
Patton et al. 2006[142]	Present results of a proficiency testing survey.	DNA sequencing	European Molecular Genetics Quality Network (EMQN)	PCR-amplified segments of cystic fibrosis (*CFTR*) gene. Wild type and common mutations were used. Primers were provided.	64	61 (95%)	36 (59%) of laboratories scored the maximum number of marks for genotyping. 53% of errors made were false-negatives (missed mutations)and 47% were false-positives (reported mutations that were not present). 27 errors of nomenclature were made.	There appears to be no correlation between the quality of the sequencing data and the likelihood of a laboratory making a genotyping mistake. Often the mutations were correctly identified in the electronic data but were not correctly identified by the interpretive software or individuals interpreting the data.

[a] Fry et al.[207] reported details on one of the three panels; Afshar et al.[143] reported details of all three panels.

Appendix D. Guidelines and Standards for Molecular Testing

Table 35. Organization Abbreviations

Organization	Abbreviation
American College of Medical Genetics	ACMG
AIDS Clinical Trials Group	ACTG
Clinical Molecular Genetics Society	CMGS
College of American Pathologists	CAP
Centers for Disease Control and Prevention – Morbidity and Mortality Weekly Report	CDC/ MMWR
Clinical Laboratory Standards Institute	CLSI
DynCorp Health Research Services	N/A[1]
European Commission	N/A[1]
European Co-operation for Accreditation	N/A[1]
European Molecular Genetics Quality Network	EMQN
U.S. Department of Health and Human Services/Food and Drug Administration	HHS/FDA
Minimum information about a microarray experiment	MIAME
National Academy of Clinical Biochemistry	NACB
National Human Genome Research Institute	NHGRI
New South Wales Department of Health	NSW Department of Health
New York State Department of Health – Wadsworth Center	N/A[1]
Organisation for Economic Co-Operation and Development	OECD
Standards Unit, Evaluations and Standards Laboratory	N/A[1]
Swiss Society of Medical Genetics	N/A[1]

[1] Abbreviations have not been used for these organizations.

Table 36. Guidelines and Standards for Laboratories Performing Molecular Tests

Organization	Title	Purpose	Description	Year	Reference
ACMG	Standards and Guidelines for Clinical Genetics Laboratories Technical Standards & Guidelines: Molecular Genetic Testing for Ultra-Rare Disorders	An overview of the specific issues that arise when performing molecular diagnosis for disorders in which the test is available in only one laboratory or very few laboratories.	Specific Issues addressed include custom mutation analysis and prenatal diagnosis, choice of analytic technique to identify private mutations, concerns about test validation, and interpretation of results.	2006	[162]
ACMG	Standards and Guidelines for Clinical Genetics Laboratories	These voluntary standards have been established as an educational resource to assist medical geneticists in providing accurate and reliable diagnostic genetic laboratory testing consistent with currently available technology and procedures in the areas of clinical cytogenetics, biochemical genetics and molecular diagnostics.	Section G: Clinical Molecular Genetics: Specifically refers to the use of molecular techniques to examine heritable or somatic changes in the human genome. Some of the topics covered include specimens & records, quality control, DNA preparation, assay validation, hybridization, PCR methodologies, controls and standards, and RT-PCR.	2006	[8]
ACTG	Virology Quality Assessment (VQA) Program Testing Requirements for New Laboratories to Obtain Approval for HIV DNA Testing in NIH-Funded Protocols	Lists requirements for HIV DNA testing approval	Areas covered include pre-certification and certification testing; re-certification; normal and fast track approval; continued approval; withdrawal/removal from proficiency program; and proficiency test panels.	2004	[151]
CAP	Laboratory Accreditation Program - Molecular Pathology Checklist	Laboratory accreditation checklist used in preparation for an inspection of a laboratory	Topics covered include proficiency testing, quality management and quality control, personnel, physical facilities and equipment, laboratory safety, and radiation safety	2007	[163]

Organization	Title	Purpose	Description	Year	Reference
CDC/ MMWR	Good Laboratory Practices for Waived Testing Sites	Summarizes study findings and provides recommendations developed by the Clinical Laboratory Improvement Advisory Committee for conducting quality waived testing.	These recommendations include considerations before introducing waived testing, such as management responsibility for testing, regulatory requirements, safety, physical and environmental requirements, benefits and costs, staffing, and documentation, and good laboratory practices throughout the testing process.	2005	[161]
CDC/MMWR	Guidelines for Laboratory Test Result Reporting of Human Immunodeficiency Virus Type 1 Ribonucleic Acid Determination	Provides guidelines for standardized reporting of viral load test results by licensed laboratories to healthcare providers and facilities for public health case reporting of HIV infection and AIDS	Topics addressed include available viral load tests, laboratory practices for HIV viral load testing, variation among laboratory viral load test reports, and recommendations for reports and documentation. Recommended standards were developed through data review, input from physicians and laboratories, and an assessment of laboratory practices.	2001	[160]
CLSI	Molecular Diagnostic Methods for Infectious Disease; Approved Guideline 2nd Edition	This guideline describes general principles for the development, evaluation, and application of tests designed for direct detection of microorganisms in clinical specimens and for identification of microorganisms grown in culture.	Addresses topics relating to clinical applications, amplified and non-amplified nucleic acid methods, selection and qualification of nucleic acid sequences, establishment and evaluation of test performance characteristics, inhibitors, and interfering substances, controlling false-positive reactions, reporting and interpretation of results, quality assurance, regulatory issues, and recommendations for manufacturers and clinical laboratories.	2006	[149]

Organization	Title	Purpose	Description	Year	Reference
CLSI	Molecular Diagnostic Methods for Genetic Diseases; Approved Guideline 2nd Edition	Provides guidance for the use of molecular biological techniques for clinical detection of heritable mutations associated with genetic disease.	Recommendations cover nomenclature for human pedigrees and the designation of mutations; laboratory safety; and "front-end" activities, such as intake information, specimen identification and accessioning, and sample preparation. Other topics addressed are molecular analytical techniques, test validation and characterization, quality assurance, results reporting, and selection of referral laboratories.	2006	[13]
CLSI	Genotyping for Infectious Diseases: Identification and Characterization; Approved Guideline	Provides guidance for the development and use of genotyping methods (DNA sequencing, single nucleotide polymorphism-SNP detection, real-time target amplification techniques of target sequences specific to particular microorganisms to identify the clinically important genetic characteristics responsible for disease manifestation, outcome, and response to therapy in the infectious disease setting.	An update on technologies used for molecular genotyping; preparation; standards, calibrators, and reference materials; analytical and clinical verification/validation; reporting and interpreting results; and the determination of clinical utility of such testing.	2006	[150]
CLSI	Proficiency Testing (External Quality Assessment) for Molecular Methods; Approved Guideline	This guideline complements currently available regulatory guidance documents regarding the management and operation of proficiency testing programs.	This document addresses people who produce, distribute, and administer proficiency testing materials. A guide for laboratories in best practices; serve as a benchmark for evaluation of new programs, and discuss assignment of target result; distribution, receipt, and evaluation of data; and reporting responsibilities.	2005	[157]

Organization	Title	Purpose	Description	Year	Reference
CLSI	Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline	This document provides guidance related to proper and safe biological specimen collection and nucleic acid isolation and purification.	Topics include methods of collection, recommended storage and transport conditions, and available nucleic acid purification technologies for each specimen/nucleic acid type.	2005	[158]
CLSI	Use of External RNA Controls in Gene Expression Assays; Approved Guideline	Provides protocols supporting the use of external RNA controls in microarray and QRT-PCR-based gene expression experiments.	This guideline addresses important issues associated with the use of external RNA controls as a tool for verification of technical performance, and in support of the evaluation of qualitative results for a specific clinical analyte including preparation of control transcripts, design of primers and amplicons, quality control, use in final experimental or clinical test application, and the analysis and interpretation of data obtained.	2006	[159]
CLSI	Verification and Validation of Multiplex Nucleic Acid Assays; Proposed Guideline	This guideline provides recommendations for analytic verification and validation of multiplex assays, as well as a review of different types of biologic and synthetic reference materials.	Topics covered include sample preparation, a general discussion of multiplex methods and technologies, reference and quality control materials, analytic verification and validation, data analysis, and reporting of results.	2007	[15]
CLSI	Immunoglobulin and T-cell Receptor Gene Rearrangement Assays; Approved Guideline	Helps laboratorians perform and interpret gene rearrangement assays.	This document includes indications for gene rearrangement analysis and acceptable methods for specimen collection, transport, and processing. Recommendations for assessing specimen adequacy, as well as technical methods for conducting gene rearrangement assays, information on sensitivity, specificity, controls, and test interpretation. Quality assurance procedures are included throughout the document.	2002	[170]

Organization	Title	Purpose	Description	Year	Reference
CLSI	Nucleic Acid Amplification Assays for Molecular Hematopathology; Approved Guideline	This guideline addresses the performance and application of assays for gene rearrangement and translocations by both polymerase chain reaction (PCR) and reverse transcriptase (RT) PCR techniques.	The topics covered include indications for molecular biologic testing, specimen collection, transport, and processing; assessment of specimen adequacy; conduct of amplification-based molecular hematology assays; sensitivity, specificity; controls; and artifacts; quality assurance; and interpretation of results.	2003	[12]
CLSI	Fluorescence *In Situ* Hybridization (FISH) Methods for Medical Genetics; Approved Guideline	This guideline has been developed to ensure appropriate and reliable use of FISH technology in medical genetics laboratories.	Provides useful recommendations for FISH assay developers, manufacturers, diagnostic genetic laboratories, and regulatory agencies.	2004	[14]
CLSI	Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine; Approved Guideline	This guideline addresses automated, PCR-based, dideoxy-terminator, and primer extension sequencing done on gel- or capillary-based sequencers.	This guideline specifies recommendations for all aspects of the sequencing process including specimen collection and handling, isolation of nucleic acid, amplification and sequencing of nucleic acids, and general interpretation and reporting of genotyping results.	2004	[171]
CLSI	Diagnostic Nucleic Acid Microarrays; Approved Guideline	This guideline provides recommendations for many aspects of the array process including: method overview; nucleic acid extraction; the preparation, handling, and assessment of genetic material; quality control; analytic validation; and interpretation and reporting of results.	The guideline addresses array-based detection variations in DNA sequence and gene expression analysis as it relates to: heritable variations, somatic changes, methylation profiling, pathogen profiling including antibiotic resistance analysis, expression profiling, and gene dosage/comparative genomic hybridization.	2006	[11]

Organization	Title	Purpose	Description	Year	Reference
CMGS	Practice Guidelines for Internal Quality Control within the Molecular Genetics Laboratory	This guideline provides recommendations for the internal quality control of testing samples.	The specific areas discussed in sample reception, recording sample data (e.g., arrival time, labeling), and DNA extraction.	2004	[152]
DynCorp Health Research Services	General Recommendations for Quality Assurance Programs for Laboratory Molecular Genetic Tests	To characterize the focus of quality assurance and proficiency testing methods in molecular genetic testing for human heritable disease, and to develop recommendations as to how molecular genetic testing can be enhanced, so that laboratory practice may more closely approximate performance goals.	Topics addressed include descriptions of technologies and current quality assurance and proficiency testing programs. Recommendations to improve the quality of laboratory practices for the benefit of public health are detailed within this report.	1999	[168]
EMQN	Draft Best Practice Guidelines for Laboratory Internal Quality Control	Provides information regarding laboratory internal quality control.	Topics covered include sample reception/storage, DNA extraction, sample handling, controls, results, reporting, documentation, audit, validation, and staff training.	2002	[34]
European Commission	Guidance Document on the Use of Reference Materials in Genetic Testing	The aim of this guidance document is to provide a short and user-friendly support to genetic testing laboratories	Topics covered include metrological context and terminology, availability of reference materials, patent issues related to reference materials, selection criteria for reference materials according to their use, and application guidance.	2008	[164]
European Commission	25 Recommendations on the ethical, legal, and social implications of genetic testing	Recommendations developed by experts from various backgrounds relevant to the ethical, legal, and social implications of genetic testing	The recommendations are organized into three categories: general framework, implementation of genetic testing in healthcare systems, and genetic testing as a research tool	2004	[166]

Organization	Title	Purpose	Description	Year	Reference
European co-operation for Accreditation	Use of Proficiency Testing as a Tool for Accreditation testing	The objective of this document is to ensure a consistent good practice for Accreditation Bodies (Abs) and laboratories in the cost-effective use of proficiency testing in accreditation.	This document is to help and unify accreditation bodies' and testing laboratories' understanding concerning the use of proficiency testing in accreditation. It also provides guidance in the use of different types of proficiency testing to support evidence of testing laboratories' competence within their scope of accreditation.	2001	[165]
FDA	Guidance for Industry and FDA Staff Commercially Distributed Analyte Specific Reagents (ASRs): Frequently Asked Questions	This guidance document is intended to clarify the regulations regarding commercially distributed analyte specific reagents (ASRs) and the role and responsibilities of ASR manufacturers.	This guidance addresses some frequently asked questions about how ASRs may be marketed, and provides FDA's Office of In Vitro Diagnostic Device Evaluation and Safety's (OVID's) and the Center for Biologics Evaluation and Research's (CBER's) responses to those questions.	2007	[33]
FDA	Draft Guidance for Industry, Clinical Laboratories, and FDA Staff - In Vitro Diagnostic Multivariate Index Assays (IVDMIAs)	Guidance addresses the definition and regulatory status of a class of In Vitro Diagnostic Devices referred to as In Vitro Diagnostic Multivariate Index Assays (IVDMIAs)	FDA seeks to dispel the existing confusion about the regulation of IVDMIAs that are developed by, and used in, a laboratory, and clarify its approach to regulating IVDMIAs.	2006	[93]
MIAME	The MIAME Checklist	Guide authors, journal editors and referees in helping them to ensure that the data supporting published results based on microarray experiments are made publicly available in a format that enables unambiguous interpretation of the data and potential verification of the conclusions.	The topics covered include experiment design; samples used, extract preparation, and labeling; hybridization procedures and parameters; measurement data and specifications; and array design.	2005	[153]

Organization	Title	Purpose	Description	Year	Reference
NACB	Guidelines and Recommendations for Laboratory Analysis and Application of Pharmacogenetics to Clinical Practice	To provide a systematic rigorous assessment of the discipline of pharmacogenetics as it applies to clinical laboratory testing and its application to clinical practice.	Issues addressed include methodological (pre-analytical and analytical) considerations, standardization and quality assurance of testing; selection of appropriate pharmacogenetics testing profiles; recommended reporting of test results and interpretation; standards needed for demonstration of clinical utility and efficacy; and, regulatory and other recommendations for effective use of pharmacogenetic information in a clinical setting.	2006	[148]
New York State Department of Health - Wadsworth Center	Clinical Laboratory Evaluation Program (CLEP) - Guide to Program Requirements and Services	CLEP has three objectives: 1) to monitor, improve, and broaden the clinical capabilities of participating laboratories and blood banks; 2) to provide guidelines, quality control standards and procedures to be used by permit-holding clinical facilities; 3) to provide continuing education opportunities for technical personnel involved in the operation of clinical laboratories through training and remediation programs.	This guide provides information for application procedures, personnel requirements, laboratory surveys, and proficiency testing.	2005	[154]
NHGRI	Promoting Safe and Effective Genetic Testing in the United States	The goal of this report is to recommend policies that will reduce the likelihood of damaging effects of genetic testing so the benefits of testing can be fully realized by undiluted harm.	This report suggests a framework for ensuring that new tests meet criteria for safety and effectiveness before they are unconditionally released, thereby reducing the likelihood of premature clinical use.	1997	[172]

Organization	Title	Purpose	Description	Year	Reference
NSW Department of Health	Genetic Testing - Guidelines for Prioritizing Genetic Tests	Developed to assist clinicians and health services to prioritize genetic test requests based on clinical need, equity of access and within available funding levels.	This guideline provides information on the NSW Department of Health priority system as a guide to appropriate genetic testing. The areas covered are prenatal, diagnostic, carrier, and presymptomatic and predictive testing.	2007	155
OECD	Guidelines for Quality Assurance in Molecular Genetic Testing	Guidelines comprise principles and best practices for quality assurance in molecular genetic testing for clinical purposes.	The guidelines seek to assist both OECD and non-OECD member countries in the development and introduction of appropriate quality assurance procedures.	2007	169
Standards Unit, Evaluations and Standards Laboratory	Good Laboratory Practice When Performing Molecular Amplification Assays	This guidance note describes key elements of how to organize facilities for polymerase chain reaction (PCR) testing including workflow, reagents, consumables, and staff.	This document describes procedures that will help minimize the carry-over of amplified DNA, and are most relevant where “in-house” assays are in use.	2006	35
Swiss Society of Medical Genetics	Best Practice Guidelines on Reporting in Molecular Genetic Diagnostic Laboratories in Switzerland	The aim of the guidelines is to improve the quality of reporting in Switzerland and to help laboratories to provide the most understandable and complete reports of their analyses.	This text presents best practice guidelines for Swiss laboratories reporting molecular genetic diagnostic testing of constitutional mutations.	2003	167

ACMG	American College of Medical Genetics
ACTG	AIDS Clinical Trials Group
CAP	College of American Pathologists
CDC/ MMWR	Centers for Disease Control and Prevention – Morbidity and Mortality Weekly Report
CLSI	Clinical Laboratory Standards Institute
CMGS	Clinical Molecular Genetics Society
EMQN	European Molecular Genetics Quality Network
FDA	Food and Drug Administration
MIAME	Minimum information about a microarray experiment
NACB	National Academy of Clinical Biochemistry
NHGRI	National Human Genome Research Institute
NSW Department of Health	New South Wales Department of Health
OECD	Organisation for Economic Co-Operation and Development