Introduction to **Molecular Diagnostics**

THE ESSENTIALS OF DIAGNOSTICS SERIES

AdvaMedDx and DxInsights Present

INTRODUCTION TO MOLECULAR DIAGNOSTICS

Abstract

Diagnostic tests are the foundation of a successful health care system, providing critical information that health care providers and patients need to make the right medical decisions. Diagnostics often provide objective, quantitative measurements that inform every stage of care—prevention, detection, diagnosis, treatment, and successful management of health conditions.

Molecular diagnostics is a dynamic and transformative area of diagnostics, leading to insights in research and treatment in many disease states that are revolutionizing health care. Molecular diagnostics detect and measure the presence of genetic material or proteins associated with a specific health condition or disease, helping to uncover the underlying mechanisms of disease and enabling clinicians to tailor care at an individual level – facilitating the practice of "personalized medicine."

Continuous innovation in technology is increasing the speed and performance of molecular diagnostics, and a future in which whole genome sequencing is routinely performed is not far away.

Increasing automation is enabling sophisticated molecular tests to be performed in the full scope of health care settings, bringing state of the art diagnostics to all areas of the world.

This report provides an overview of the current landscape for molecular diagnostics, explains the key technologies that are driving the molecular revolution, illustrates the power of molecular diagnostics with some specific examples, and concludes by noting several challenges that have the potential to influence progress in this critical field of medicine.

Table of Contents

Introduction to Molecular Diagnostics

THE ESSENTIALS OF DIAGNOSTICS SERIES

Introduction

Diagnostics are an integral and critical part of our health care system, as the results of these tests inform a wide range of medical decision making. From the genetic tests that inform personalized cancer treatment to the microbial culture that identifies the right antibiotic to fight an infection, diagnostic tests provide critical insights at every stage of medical care—prevention, detection, diagnosis, treatment and successful management of health conditions. Figure 1 illustrates the roles of diagnostics throughout this continuum of health care.

Comprehensive Role of Diagnostics

Diagnostics can help clinicians optimally manage patients through the continuum of care.

	Risk Assessment	Screening	Diagnosis	Staging and Prognosis	Therapy Selection	Monitoring
Description	Diagnostic tests to complement traditional risk factors	Applied to high-risk patient to identify disease early	Use for definitive diagnosis and general typing	Assess severity and/or risk of recurrence Inform adjuvent therapy decision	Used to predict efficacy or safety response to specific treatments	Recurrence monitoring Monotoring for treatment efficacy
Clinical Implications	Implement wellness programs proactively	Nip disease in the bud with early treatment	Refer to the appropriate specialist	Determine whether treatment is necessary	Do not waste unproductive therapy	Control disease progression with changes in treatment

SOURCE: DxInsights White Paper January 2012

FIGURE 1: Role of diagnostics through the continuum of health care.

Broadly speaking, two primary areas of health care diagnostics are "in vivo" imaging and "in vitro" diagnostics, often referred to as IVDs. Imaging encompasses such technologies as X-Rays, ultrasonic waves, magnetic resonance, or radio-nuclear methods that produce images of the body and its organs and other structures. IVDs are tests performed on a sample taken from the body (blood, tissue, sputum, urine, etc.). "In vitro" is from Latin, meaning "on glass", in reference to the glassware used to hold bodily samples during culture or examination. Molecular diagnostics, the topic of this report, is a subset of in vitro diagnostics, and, therefore, the scope of this report is confined to this field, which is hereafter referred to as "diagnostics" for the sake of convenience.

While the traditional laboratory remains a mainstay for diagnostic testing, significant testing is done outside the laboratory, in such point of care settings as hospitals, physicians' offices, and clinics, and for personnel in the field, such as emergency responders and soldiers. Pregnancy tests and diabetes test strips are familiar examples of diagnostics that are available directly to consumers.

The main categories of diagnostics are clinical chemistry, immunology, hematology, microbiology and molecular diagnostics. The diagnostics industry continues to innovate in all of these important areas, and molecular diagnostics has captured particular attention in recent years because of the deep insights these types of tests bring to diagnosis and treatment.

Molecular Diagnostics

Molecular diagnostics is one of the most dynamic and transformative areas of diagnostics, leading to advances in research and treatment that are revolutionizing health care across a wide range of diseases and health conditions.

"Molecular diagnostics" is a broad term describing a class of diagnostic tests that assess a person's health literally at a molecular level, detecting and measuring specific genetic sequences in deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) or the proteins they express. Molecular diagnostics identify gene, RNA, and protein variations that shed light on whether a specific person is predisposed to have a disease, whether they actually have a disease, or whether a certain treatment option is likely to be effective for a specific disease. These tests also can detect and quantify the presence of specific viruses, bacteria, or types of cells.

Sequencing the entire human genome is a feat that, when it was first accomplished by the Human Genome Project, took an international, government-led research consortium over 10 years and $2.7 billion. (An initial draft of the entire genome sequence was published in 2001, ten years after the project was initiated, and an essentially complete version was published in 2003.) Remarkably, molecular diagnostics now can sequence a person's entire genome for a cost that is rapidly declining and now approaches $1,000.

Many of our bodily processes, both normal and abnormal, as well as health or disease states, are driven by the interaction of our genes and the proteins they produce that carry out specific functions within the body. Therefore, the ability to quickly and accurately assess an individual's health at this molecular level is truly transforming the practice of medicine.

The term "personalized medicine" has arisen from this field of molecular discovery. The term means that understanding underlying molecular mechanisms is empowering clinicians to evolve away from treating every patient based on what they have broadly in common with other patients (e.g., lung cancer) to treating them as individuals (e.g., a patient has a specific gene mutation in their cancer that is associated with a specific type of lung cancer).

Of course, the ultimate power of personalized medicine is the ability to treat these smaller groups with therapies tailored to the molecular profile of their individual cancer. When specific proteins or genetic sequences have a known association with a specific health condition or disease, they are often referred to as "biomarkers"" because they are markers of that condition or disease.

Molecular diagnostics are the tools that are driving the continuing discovery of biomarkers at the research level, which in turn leads to treatments designed around these biomarkers. Then molecular diagnostics play an additional critical role by ensuring that these new therapies are delivered to the right patients through more accurate diagnosis of the exact nature of their individual disease. This has led to the emerging field of companion diagnostics, in which a molecular diagnostic test is used to identify whether a specific therapy (a companion to the diagnostic) is likely to be effective for an individual patient.

Molecular diagnostic tests can help a woman understand the likelihood that her breast cancer will reoccur later in life, or tell a doctor what drug is the right treatment for a late-stage melanoma patient. They can make it possible for couples considering a family to know if they are carriers of a cystic fibrosis gene mutation and therefore at risk of having a child affected by cystic fibrosis. Molecular diagnostics can identify multiple strains of respiratory viruses in a single test, or monitor the level of HIV virus in a patient's blood to determine how well their treatment is working. In these and many other ways, molecular diagnostics are transforming health care.

Molecular diagnostics today are routinely used in hospitals, reference labs, and blood banks. In the latter, molecular tests are used to screen donated blood products for infectious diseases like hepatitis and HIV. In hospitals, testing is often performed to identify pathogens in patients with infections. In fact, infectious diseases are one of the strongest growing areas within the molecular diagnostics field. There is also increasing demand for such technologies at

the point of care (testing that occurs at the point of treatment or patient interaction with a health care provider, such as a doctor's office or a clinic).

There are far too many types of molecular diagnostic tests to cite in this report, including thousands of hereditary genetic tests, and the field is rapidly evolving. The report that follows provides an overview of the current field for molecular diagnostics, explains the technology that is driving the molecular revolution, illustrates the power of molecular diagnostics with some specific examples, and concludes by noting several challenges that may slow progress in this critical field of medicine.

The Molecular Diagnostics Field

The overall global market for diagnostics was valued at $45.6 billion in 2012 and is expected to grow at about 7% annually over the next five years to reach a market size of $64.6 billion in 2017[1]. The United States and Europe account for about 60% of that market, with Asia Pacific forecast as the highest growth region over the next five years.

The U.S. diagnostics market was about $15.5 billion in 2012, with a forecasted growth rate of 5.8% over the next five years.

In developing countries, growth is propelled largely by increasing access (often meaning populations getting access to meaningful health care for the first time) and increasing government investments in health care. A recent example of a test that is meeting this need is a new MTB/RIF assay that reduces the time to diagnosis of tuberculosis/rifampin resistant TB from 8 weeks to 2 hours and can be done "in the field", in a mobile van, powered by solar energy. While there continues to be growth in developed markets as well, it is tempered by a perfect storm of constrained health care budgets, regulatory hurdles, and increasing evidence expectations from payers.

Molecular diagnostics is currently a relatively small portion of the overall diagnostics market ($5 billion, or about 11%), but the fastest growing segment (See Figure 2). Globally, the molecular diagnostics market is estimated to grow over 12% annually through 2017. Today, molecular diagnostics is employed primarily in developed markets (with the United States and Western Europe accounting for about 80%), where most of the five-year growth is expected to take place.

Currently, molecular diagnostic revenue is dominated by infectious disease testing (50-60%), with about another third attributable to genetic testing, and blood screening applications accounting for much of the rest. For the reasons discussed earlier in this report, growth in the molecular diagnostics market is spurred by the excitement over its potential to revolutionize areas across the value chain in health care.

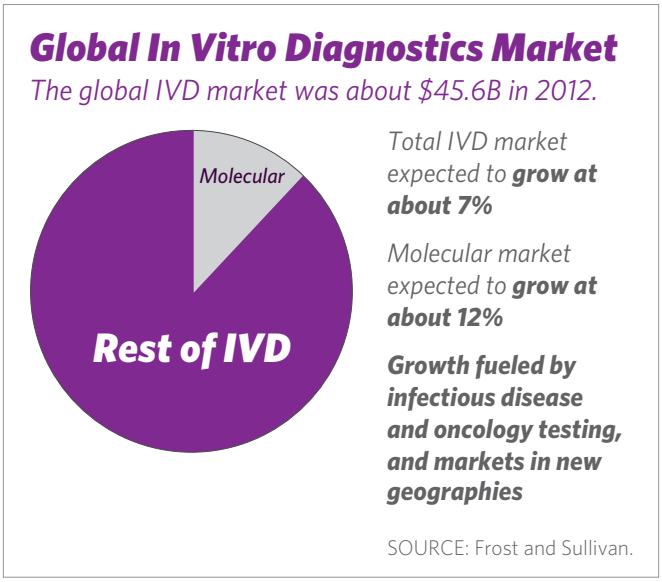


FIGURE 2: Molecular Diagnostics as a component in the global in vitro diagnostics market.

For example, in pharmaceutical industry research, understanding the mechanism of diseases is critical for discovering the next breakthrough targeted therapies. Identification of patients with a specific genetic profile (hereditary or somatic) or signature that predicts response to a specific drug or dose is essential to increasing the proportion of patients with favorable outcomes, thereby enhancing the overall effectiveness of the therapy in the treated population.

Indeed, the FDA has stated that when a new therapy is developed for which there is a diagnostic that provides essential information for its safe and effective use, it will require concurrent approval or clearance of the

[1] Market statistics from Frost and Sullivan.

diagnostic. It underscores that the development of these pharmaceuticals and companion diagnostics is a key area of market growth.

For pharmaceuticals already on the market, the development of new companion diagnostics that can better guide the use of those pharmaceuticals is another area of growth.

Molecular Biology – *Genomics and Proteomics*

The ability of a health care practitioner to efficiently deliver effective care depends upon their ability to accurately identify the cause of the patient's problem, i.e., make the diagnosis. Over time, our understanding of the mechanistic basis of disease has increased and furthered our ability to make more accurate and specific diagnoses.

Early healers needed only to feel the patient's skin to diagnose "fever," for example, but with further understanding of different illnesses and their symptoms, doctors gained the ability to distinguish between yellow fever, scarlet fever, bubonic fever and other types of infections.

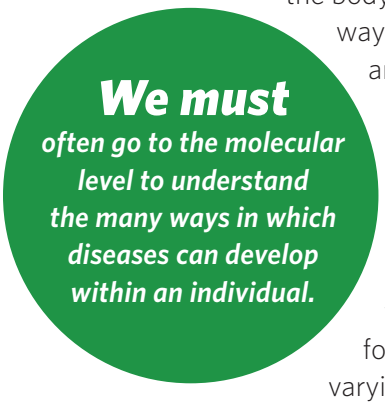
There are records of the study of gross anatomy and pathology (organs and tissues) dating back to at least the ancient Greeks. It was the advent of modern microscopy and histopathology, or cellular pathology, in the nineteenth century, however, which enabled a tremendous leap forward in our understanding of the biology of disease at a cellular level. It became possible to view infectious microorganisms in samples taken from the body, and it was further discovered that the cells of some different types of microorganisms could be distinguished by whether or not they absorbed certain dyes or stains.

Initially, different stains were used to visualize different types of cells and bacterial structures to help both see and identify specific microorganisms. Danish scientist Hans Christian Gram described this differential staining technique in 1884, and, in recognition of his work, the terms Gram-negative (failure to stain) and Gram-positive (stain absorbing) were coined to describe different categories of bacteria. Eventually, it was realized that the same cell wall characteristics that made Gram-positive cells absorb dye also made them more susceptible to certain antibiotics. The Gram stain gave diagnostics a new and prominent role in guiding therapeutic decisions.

The Gram stain remains a widely used diagnostic test due to its ability to quickly identify the presence of infection, as well as aid doctors in making a quick decision regarding antibiotic treatment. It remains only a first-line tool, however, with significant limitations. Today, cell cultures, additional antibiotic sensitivity testing, and genetic information from molecular diagnostics provide critical additional information.

With the advent of the field of molecular biology, scientists began isolating and studying genes and biochemicals that serve as biomarkers in healthy and diseased human cells and tissues. Our modern microscopes and electron microscopes gave us the ability to look at cells, their proteins, and other subcellular structures to see changes associated with diseases. We began to understand disease resistance and immunity and why certain people developed diseases and others didn't.

But how do we learn about the mechanisms that are the root causes of diseases? We must often go to the molecular level to understand the many ways in which diseases can develop within an individual. Our DNA is the inherited material found in almost every cell of the body, which governs the way our bodies develop and function.



DNA is composed of two interlocking, helical strands (the famous double helix), each of which is a string of four chemical bases in varying sequence: adenine (A), guanine (G), cytosine (C), and thymine (T). Adenine on one strand is paired with thymine on the opposite strand, and guanine is paired with cytosine, forming what are referred to as "base pairs". Reference is often made to "nucleotides", which are the basic structural building blocks of DNA (and

RNA), consisting of a base combined with a sugar and a phosphate group. A strand of DNA, therefore, is a chain of nucleotides. The terms "nucleotide" and "base" are often used interchangeably in reference to pieces of a DNA sequence.

Each complete human DNA helix contains a sequence of about three billion base pairs. The sequence of the bases is more than 99% identical in all people. The one percent of sequences that are variable is responsible for the differences that occur among humans. Our genes, which are the functional units of DNA, are segments of the DNA strand that range in size from a few hundred bases to more than 2 million bases.

Genes produce RNA (ribonucleic acid) through a process called transcription, which in turn directs the production of the proteins that make up the machinery of our cells—and, consequently, the structures and functions of our bodies. We refer to this protein production as the "expression" of the underlying genes. The complex specialization, or differentiation, of cells throughout the body is a result of only specific genes being active in certain cells at certain times, and therefore expressing proteins related only to the functions of those cells. Differences in genes among individuals, and the differential expression of those genes in a given individual, account for the physiological diversity of our race, as well as many of our diseases and health conditions.

An essential cellular function is DNA replication, in which the helix separates, each strand is duplicated, and then the duplicate strands combine through base pairing. This is how an exact copy of an individual's DNA is transferred from one cell to another during cell division so that every cell contains the same DNA.

The study of the activities and interactions of our genes is referred to as genomics, and the related study of protein activity is referred to as proteomics. In these fields of study, molecular diagnostics examine our DNA, RNA, and proteins to assess how they are functioning. Diseases disrupt the function of cells and, therefore, can be detected and/or monitored by examining the molecular alterations of DNA, RNA, or the proteins in patients' tissues, fluids, or tumors, or in the infectious agents themselves (e.g., viruses or bacteria) that cause the disease.

A particular gene whose progression of base pairs is typical of the population at large is often termed the "wild type" or "normal" gene sequence. Gene variants that are deviations from the wild type may or may not be clinically significant and extensive human research is often needed to determine their relevance. A gene variant present in more than one percent of the population is called a polymorphism and may be considered a normal variant.

There are three kinds of gene mutations, which may be a change of only one base or a long sequence of bases. These are (1) hereditary – a mutation that is passed from parent to child; (2) de novo – new mutations that arise in the egg or sperm cell, or shortly after fertilization, and so are repeated throughout the body; or (3) somatic – those that arise during life from environmental causes or through an error in DNA replication, and are typically present in tumor cells.

When specific mutations, or sets of mutations, are known to be biomarkers associated with a disease or condition, molecular diagnostic tests can examine a patient's genes to determine whether those mutations are present. These tests may look only for those certain gene variants, or map the entire sequence of a targeted portion of DNA to detect all mutations in the sequence.

Depending on the nature of the biomarker in question, molecular diagnostics therefore can assess a person's risk of developing a disease, determine whether a person is a carrier of a hereditary condition, screen for diseases that are present but not yet symptomatic, or provide a diagnosis of existing symptoms.

Molecular Diagnostics

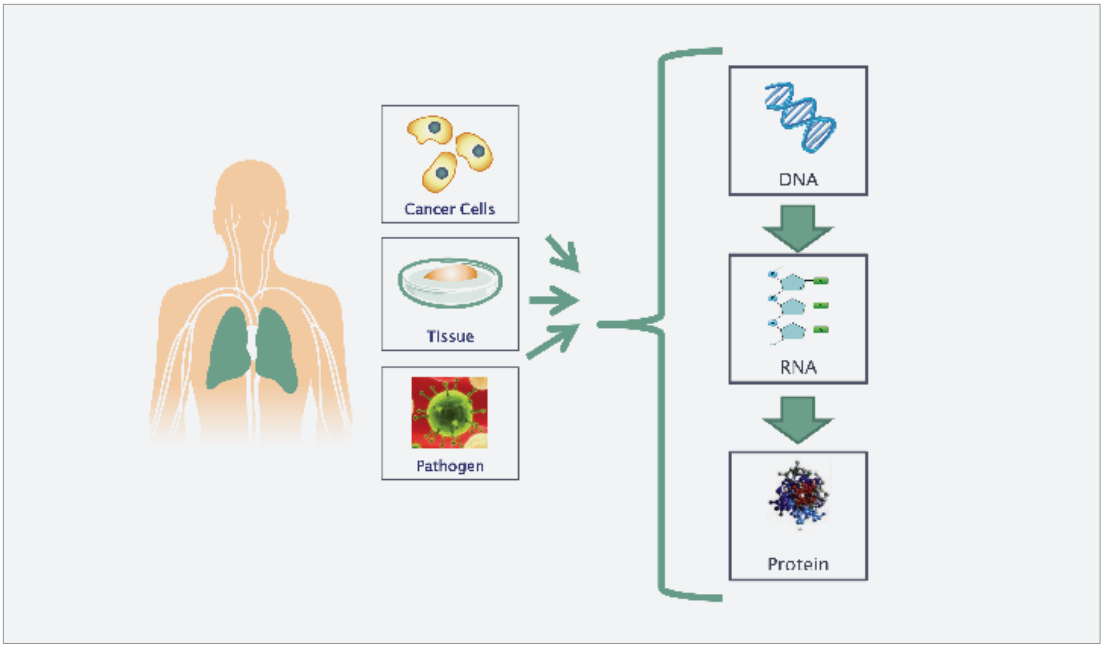


FIGURE 3: Molecular diagnostics examine the molecules in the cell, i.e. the DNA, RNA or proteins, and how their role in human biology and disease.

Technology of Molecular Diagnostics

A number of techniques are employed in modern diagnostics to detect and quantify specific DNA or RNA sequences, as well as proteins. This report provides a summary overview of one of the most fundamental of those techniques, the polymerase chain reaction, that is used to amplify specific sequences of DNA or RNA. Subsequent reports will elaborate on additional genetic test methods, such as in situ hybridization or whole genome sequencing, and such protein detection tests as mass spectrometry. Some of these additional technologies are described briefly below:

- In situ hybridization (ISH or FISH): a technique that "unzips" DNA or RNA in the sample and uses a so-called probe—a labeled DNA or RNA strand that hybridizes with the target, complementary sequence on an unzipped strand and thereby identifies and quantifies the target sequence in the sample. FISH stands for Fluorescent In Situ Hybridization due to its use of a fluorescent probe that facilitates automated reading of the results.
- Chips and Microarrays: these technologies simultaneously measure RNA or cDNA (the expression of a large number of genes), or DNA (single nucleotide polymorphisms (SNPs), or genome regions).
- Mass spectrometry (MS): a technology that determines the molecular mass of a charged particle by measuring its mass-to-charge (m/z) ratio. This technology is used to find and analyze protein based biomarkers and is broadly classified into gel-based and gel-free techniques.
- Sequencing (CE, NGS): a technique used to map out the sequence of the nucleotides that comprise a strand of DNA. Today, this can be done via capillary electrophoresis (CE) or through multiple next generation sequencing (NGS) methods.

Molecular Diagnostics

DNA Sequence Detection using Polymerase Chain Reaction (PCR)

One of the essential methods underlying many molecular diagnostics is amplification, the process of making copies of a specific DNA or RNA sequence found in a sample (e.g., blood or tumor) until there are so many copies that they can be detected and measured.

There are a number of different amplification technologies, but polymerase chain reaction (PCR) testing is the most widely used and is considered a work horse in molecular diagnostics. Chemist Dr. Kary Mullis received a Nobel Prize for inventing PCR in 1983.

PCR is a powerful tool for locating short segments of a gene where known critical mutations or variances can lead to altered cell functions associated with disease or altered function. PCR tests for the presence of a portion of DNA that has a known base sequence, employing the same enzymatic process used by natural DNA replication to rapidly amplify, or copy, that sequence until there are thousands or millions of copies. Because PCR relies on amplification, it is highly sensitive, meaning it can detect specific DNA segments that may be present at very low levels in the sample.

Early researchers developed ways to detect the presence of PCR-amplified DNA by how far it traveled in a gel in relation to other, different-sized, pieces of DNA when all the pieces were pulled by an electric current (gel electrophoresis). Gel electrophoresis can still be used, but now the use of fluorescent dyes, that bind to the target DNA segment as it is amplified, enable automated instruments to monitor the resulting fluorescence without using gels. In this manner, technologies such as quantitative real-time PCR not only can detect the presence of the target DNA segment, but also quantify the amount of target DNA that was originally present in the sample. This can be used, for example, to determine the prevalence of an infectious agent in the body.

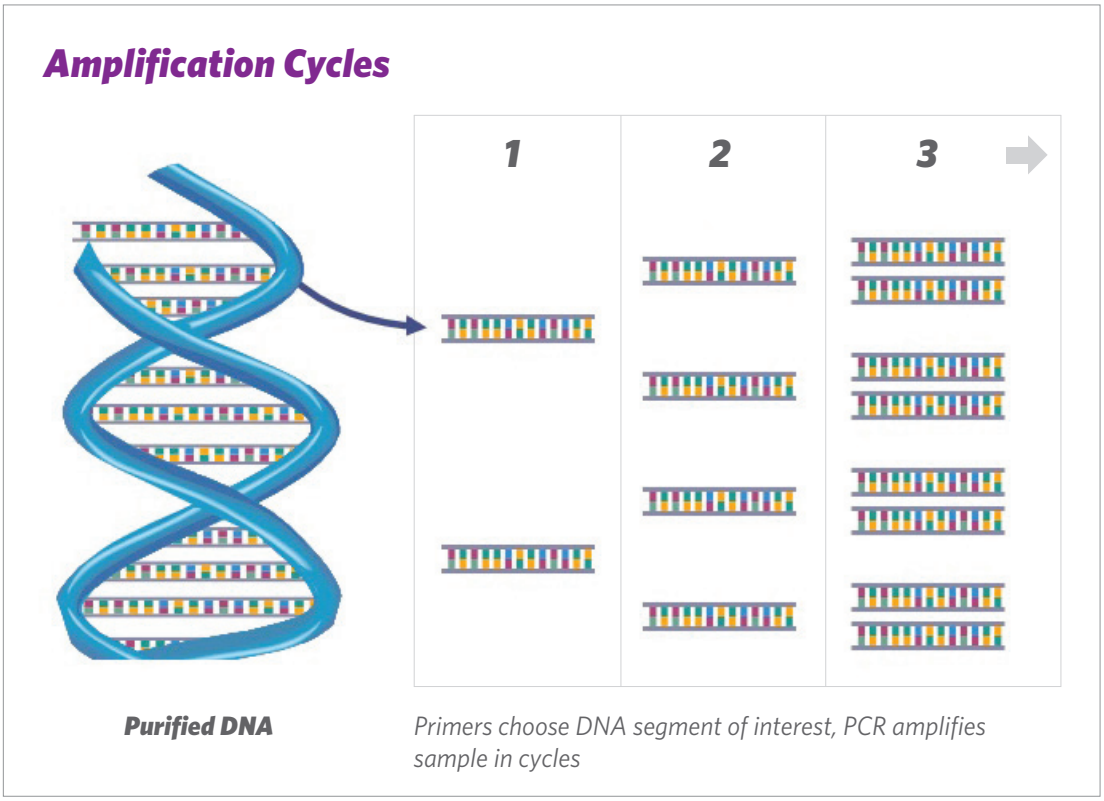


FIGURE 4: Polymerase Chain Reaction to amplify DNA by repeating cycles that each duplicates the base sequence in a specific section of the DNA strand.

Molecular Diagnostics

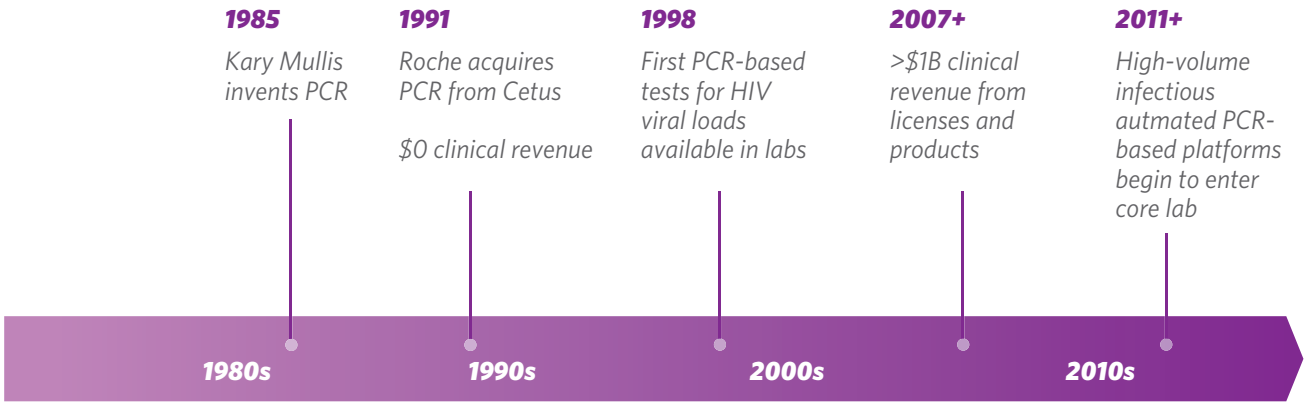
Over time, the use of PCR evolved from diagnostics for single gene mutations, to amplification-based HIV viral load testing, to multiplex testing (testing for multiple sequences at once), to high volume infectious disease testing. Most PCR tests used chemical reactions that rely on rapid cycles of temperature change to generate new strands of DNA. First, the DNA must be extracted and purified from the raw human sample (e.g. blood, tissue, saliva, etc). This is done using various scientific methods depending on the type of sample. For example, to extract DNA from blood, the blood is separated to isolate the cells from surrounding fluids, is suspended in a solution to stabilize the cells and the cells are then broken open with detergents. Additional chemicals are added to remove proteins and to keep the DNA from breaking apart. Finally, the DNA is separated from all of the other products in a series of separation steps. Several life science companies have developed easy-to-use kits for the purification of DNA that combine many of these steps, so it is not as complex and "hands-on" as it was a decade ago.

Then, to perform a PCR test, a researcher or laboratorian will add multiple chemicals to a small tube or well in a plate. These chemicals include buffer, a mix that contains the building blocks of the new DNA strand called dNTP mix (dATP, dCTP, dGTP, DTTP), salt (i.e., magnesium chloride or MgCl2), and primer mix. Primers are short pieces of DNA that have a complementary sequence to the particular target gene sequence of interest, which specifically identifies the target, out of the whole DNA sample, and ensures replication of that sequence of interest. Then, the purified DNA is added along with water, and an enzyme called TAQ polymerase. TAQ is an enzyme - a thermostable DNA polymerase - which drives the amplification reaction by adding bases to the primer to replicate the target sequence. The total volume in the tube can be as little as 20 microliters, or less than a drop of water. The tube or plate is sealed and placed into a PCR thermocycler, which is an instrument that rapidly heats and cools the amplification reaction to mimic DNA replication much faster than it occurs naturally. In non-automated PCR systems, once the DNA is amplified, a small aliquot is taken from the tube and checked to make sure that the DNA size is as expected after the reaction. The amplified DNA can then be analyzed in multiple ways depending on the desired result. The DNA can be measured just for size to check for large deletions or insertions of DNA, it can be measured for abundance, or it can be further prepped and sequenced to determine, nucleotide by nucleotide, what the DNA sequence in the sample is. In automated PCR systems, dNTPs can be tagged with dyes or other chemicals that can be detected by the instruments as amplification occurs ("real-time PCR"), without requiring intervening manipulation by a laboratory technician. The output is generally an amplification curve.

Researchers continue to devote resources to simplifying and decreasing the time for the PCR process. Some are working on decreasing the need for the upfront DNA purification before the reaction, and/or working on amplifying in less time. Finally, newer isothermal methods that do not rely on temperature cycles have simplified testing equipment and allowed versions to be produced for use outside the laboratory and even in quite elementary health care settings. Whereas PCR based diagnosis used to take days or weeks to perform by a trained molecular biologist, some systems now can produce a patient result in as little as 30 minutes by non-laboratory personnel at the point of care.

Companies have worked diligently to develop PCR and other amplification methods into the most powerful clinical diagnostic technologies to date. The transition of these molecular diagnostics from highly specialized, low volume laboratories to high volume core lab assays, and to point of care testing, has taken place quite rapidly.

Molecular Diagnostics



NOTE: Adapted from Health Advances AACC presentation by Kristin Pothier 2011
SOURCE: Washington G2 reports, Health Advances interviews and analysis, Roche company data.

FIGURE 5: Adoption and impact of PCR.

The Clinical Role

Diagnostics are important decision making tools for every stage of care – risk assessment, screening, diagnosis, staging and prognosis, therapy selection, and monitoring. There are far too many molecular diagnostics in use to cover in this report, but Figure 6 provides an example of a molecular diagnostic being used in each of these stages. This section goes on to discuss in more detail several examples of how molecular diagnostics are making a critical difference in patient care.

Molecular Diagnostics Examples

There are hundreds of examples of molecular diagnostics across this continuum.

	Risk Assessment	Screening	Diagnosis	Staging and Prognosis	Therapy Selection	Monitoring
Description	Diagnostic tests to complement traditional risk factors	Applied to high-risk patient to identify disease early	Use for definitive diagnosis and general cancer typing	Assess severity and/or risk of recurrence Inform adjuvent chemo decision	Used to predict efficacy or safety response to specific treatments	Recurrence monitoring Monotoring for treatment efficacy
Molecular Diagnostic Example	CF carrier testing BRCA1 testing	MRSA HPV	TB CT/NG	OncoType Dx MammaPrint	BRAF KRAS Her2	BCR-ABL HIV viral load

FIGURE 6: Examples of molecular diagnostics across the continuum of care.

Molecular Diagnostics

Screening Test Example: HPV

The fight against cervical cancer is largely a success story in the United States and other developed countries, where the introduction in the 1940s of the Papanicolaou (Pap) smear test to look for abnormal/precancerous cervical cells has driven down death rates from cervical cancer by as much as 70 percent. The CDC reports that cervical cancer affects about 12,000 women in the United States each year[2], however, and is the second leading cancer of women worldwide, due to poorer access to health care in developing countries. Globally, cervical cancer affects about 500,000 women and kills about 250,000[3].

The most successful treatment of cervical cancer is delivered as a result of early detection. The use of HPV tests to identify women most likely to develop cervical cancer has the potential to revolutionize treatment of this disease.

About 20 years ago, researchers discovered a link between the human papilloma virus (HPV) and cervical cancer. It is now known that, of the 100 or so genetically distinct types of HPV, about fourteen are associated with a high risk of cervical cancer, and just eight of those are responsible for almost all cases.

Because HPV types are genetically identifiable, it has been possible to develop molecular diagnostic tests to screen women for the high risk types. Identifying women with these high risk types enables those women to schedule more frequent exams and can guide a physician to follow up more aggressively when a Pap test yields abnormal or inconclusive results. In this way, molecular diagnostics are driving the next advances in early detection and prevention of cervical cancer after the historical success of the Pap test. The American Cancer Society and other leading professional societies recommend that women 21-65 years old should receive a Pap test (or other form of cytological test) every three years, and women 30-65 years old should receive a cytological test together with an HPV test every five years.

Screening/Diagnostic Test Example: Cystic Fibrosis

The cystic fibrosis test is an example of a molecular diagnostic test that can be classified as a screening test or a diagnostic test, depending on when it is used. Cystic fibrosis (CF) is an inherited chronic disease that mainly affects the lungs and digestive system and currently afflicts about 30,000 children and adults in the United States (70,000 worldwide)[4]. About 1,000 new cases are diagnosed each year.

CF is caused by an abnormality in the CF transmembrane regulator (CFTR) gene that prevents the normal movement of chloride ions across membranes. The disease is characterized by a buildup of mucus in the lungs, pancreas, and other organs of the body. Over time, this results in patients having serious breathing and digestive difficulties and an average life expectancy that is less than 40 years.

CF is seen in patients with two altered CFTR genes, one from each parent. Those that only have one copy of the altered gene are considered "carriers", and while they do not have symptoms of CF, they can pass the gene to their children. There are about ten million carriers in the United States. Therefore, "carrier screening" of parents-to-be or pregnant women determines their CFTR gene status and helps families adequately prepare for the results. Testing is also done on amniocentesis samples to directly assess CF status in the unborn child. Newborns are screened in all 50 states to assess CF status.

One of the most common mutations causing CF is the delta F508 deletion mutation which can be detected using a number of molecular techniques. These tests use DNA amplification methods to amplify key portions of the CFTR gene and look for the mutations that cause CF.

[2] www.cdc.gov [3] www.path.org [4] www.cff.org

Molecular Diagnostics

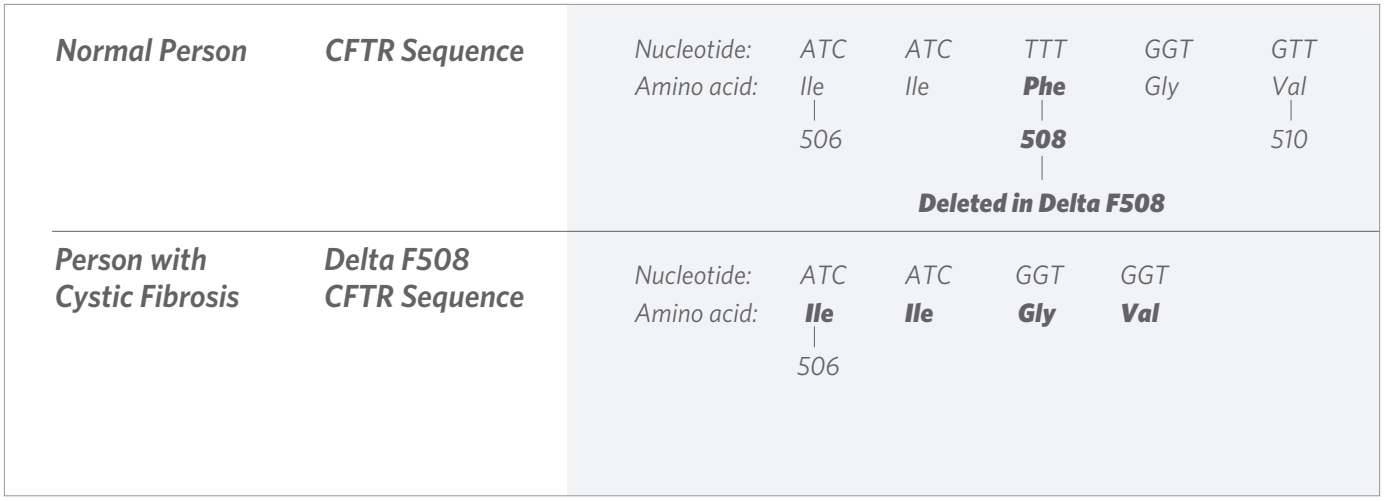


FIGURE 7: Genetics in Cystic Fibrosis: A person with cystic fibrosis has a deletion of three nucleotides in the CF gene which results in deletion of one amino acid in the CF protein and the classic CF symptoms.

The American College of Obstetricians and Gynecologists (ACOG) and the American College of Medical Genetics (ACMG) recommend offering testing for 23 common CFTR mutations to all women currently planning a pregnancy or seeking prenatal care. This helps identify carriers and thereby informs their reproductive planning. Testing can also be done on the newborn or later in childhood to diagnose CF.

Diagnostic Test Example: Chlamydia/Gonorrhea

One of the most common infectious disease tests is the molecular test used to detect chlamydia and gonorrhea (a CT/GC test). Because co-infection is common, a combined molecular test allows for amplification and detection of both *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) DNA.

Chlamydia is the most frequently reported bacterial sexually transmitted infection in the US, with over 1.4 million diagnosed and an additional 1.4 million people with the disease who are not diagnosed. Gonorrhea is also common, with an estimated 700,000 with the disease but less than half actually reported[5].

These sexually transmitted diseases are caused by bacteria that can be detected through urethral or cervical samples (as well as a urine sample for gonorrhea) using molecular testing. These tests allow physicians to quickly and definitively diagnose CT/GC and prescribe antibiotics to the patient, clearing their infection and equally importantly, stopping spread of the diseases. A rapid (90 minute) molecular CT/GC test for use at the point of care has recently been developed, providing patients with an immediate diagnosis and helping to ensure appropriate follow up.

Therapeutic Decision-Making Example: KRAS

An example of a molecular diagnostic that improves therapeutic decision-making is K-Ras testing. K-Ras is a protein, encoded by the KRAS gene, that plays a critical role in cell division, cell differentiation, and the self-destruction of cells. KRAS mutations that produce an abnormal, overactive K-Ras protein are found in pancreatic, colorectal, lung and other cancers.

Detecting a KRAS mutation is used to determine patient suitability for certain therapies in colorectal and lung cancer. Anti-EGFR (epidermal growth factor receptor) drugs (Erbitux (cetuximab) and Vectibix (panitumumab)) are key therapies for those cancers, but patients whose cancers contain KRAS typically fail to respond to these anti-EGFR therapies. Therefore, Erbitux and Vectibix labels recommend against prescribing the drugs for patients with certain KRAS mutations. KRAS mutation testing is typically performed for patients with metastatic colorectal

[5] www.cdc.gov

cancer or metastatic lung cancer via direct DNA sequencing or PCR and other amplification-based methods.

KRAS mutation testing is just one of a growing number of examples of molecular diagnostics that help inform a physician's decision making regarding appropriate treatment. Identifying which patients are unlikely to respond to a specific kind of therapy reduces the costs of ineffective treatment (measured in dollars, unnecessary side effects, and time that could be spent on other treatments).

Therapeutic Monitoring Example: HIV

Finally, many molecular diagnostics improve monitoring and management of disease. Some of the most common monitoring molecular tests are tests for HIV viral load in patients affected with HIV. HIV viral load tests are amplification-based tests that measure the amount of HIV RNA to determine how many copies of the virus are present (measured in copies per milliliter of blood). Keeping viral levels as low as possible is key to staying healthy with HIV.

Because researchers and physicians have made great strides in the care of HIV patients in the three decades since HIV was first detected, essentially transitioning it into a chronic disease in many developed countries, the HIV viral load test has become an essential tool as patients cycle through different antiretroviral therapies and develop resistance to certain therapies. Periodic HIV viral load tests indicate whether or not the disease is progressing, either because an untreated patient's immune system can no longer suppress replication of the virus, or because the therapy for a patient in treatment is no longer working.

The HIV viral load test is not used for initial diagnosis (this is often done with antibody tests), but typically also is used upon initial diagnosis as a staging diagnostic, to get a first report on the progression of the disease prior to treatment.

The Future of Molecular Diagnostics

This report includes only a few of the hundreds of examples in which the latest molecular technology is being used for rapid diagnosis and to aid physicians in improving clinical outcomes for their patients. Since we are only in the early stages of understanding the complex fields of genomic and proteomics, however, and diagnostic technologies are evolving rapidly, it seems fair to say that much of the promise of molecular diagnostics is still ahead of us.

As we understand more about the complexity of diseases, there are an increasing number of molecular biomarkers that must be identified to fully characterize the many subsets of populations that have highly variable symptoms and clinical courses. New technologies are making it possible to gather more information to more fully characterize the disease state, the best treatment alternatives for a patient, or in the case of infectious disease, rapidly identify a specific pathogen out of many that could be responsible for a particular infection (e.g., respiratory viruses). Approaches that may address this need for increasing amounts of data include multi-marker or multiplexed approaches (methods that can test for multiple biomarkers in a single assay) and gene sequencing that can detect multiple defects associated with a particular subset of patients with a disease. Practical application of these methods can be challenging, due to complexity of the assays involved, but these challenges are being met by integration of molecular methods with advanced information technology (IT) and data analytic methods.

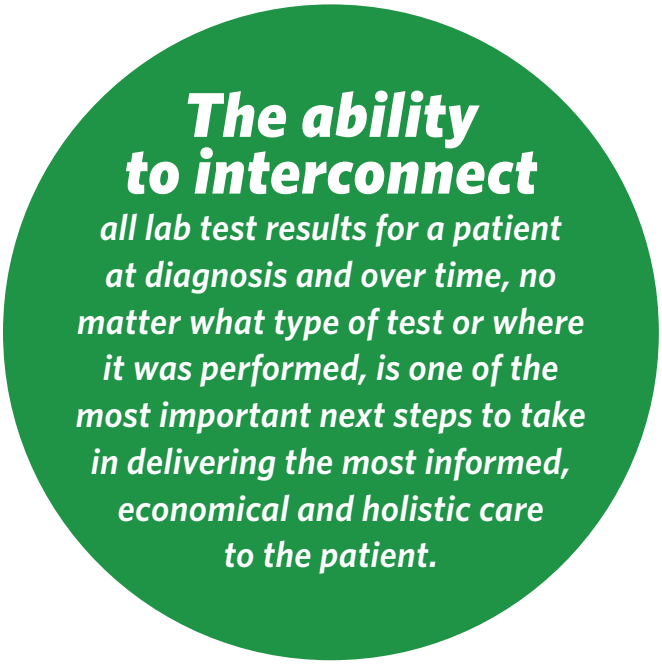
New technologies also will bring greater efficiency to current methods. There has been a great deal of excitement about the promise of nanotechnology to support the development of the molecular diagnostics field. Companies have commercialized technology based on gold nanoparticles that allow increased sensitivity and specificity for molecular diagnostics assays and that also enable multiplexing of assays. Other nanotechnology-enabled approaches include innovative ways to sequence DNA in which a single DNA strand is extruded through a nanopore and the sequence is "read" base pair by base pair.

Molecular Diagnostics

Finally, molecular diagnostics at the point of care is making great strides. Advancements have focused on ease of use, producing tests that can be performed in less specialized laboratories, rapid turn-around time, and appropriately small size and simplicity for resource-constrained settings.

Molecular diagnostics are truly transformative technologies. Yet excitement about the future of this field must be tempered by recognition of the challenges of continuing innovation and timely patient access. The challenges include regulatory hurdles, coverage and reimbursement issues, and practical and ethical (privacy) issues associated with generating and storing large volumes of highly detailed and personal health information.

- The fast pace of technological development in molecular diagnostics, and the rapidly growing body of research regarding biomarkers, challenge regulatory bodies to stay current. Clear understanding of regulatory expectations for multiplex tests, companion diagnostics and next generation sequencing as well as the increasing investments necessary to generate the clinical evidence required for approval or clearance will be integral.
- There are questions regarding the patenting of gene sequences currently working their way through the courts.
- Insurance coverage and payment for diagnostics is under constant downward pressure as payers try to keep down escalating health care costs. Despite the strong value proposition of diagnostics overall, payers are increasingly asking for health economic evidence supporting the use of specific tests, which often requires further evidence generation above and beyond regulatory requirements.
- The ability and capacity to capture, store and analyze the tremendous and rapidly increasing amount of diagnostics data, particularly with gene sequencing tests, is an ongoing challenge. IT systems must have enough power to process and analyze these huge volumes of information. In addition, another key challenge is the need for data standards that would speed interpretation of these complex genomic data sets and make the results relevant for specific patients. This task is further complicated by the fact that the patient's medical record is often in many unconnected places, whether in multiple out-patient offices, multiple hospitals, or multiple pharmacy systems.
- Finally, ethical questions are also being raised about the disclosure and use of genetic information. Genetic markers are being identified that can signal the propensity of a patient to develop a disease over their lifetime and the question has been raised of how, if at all, such information should be disclosed, particularly in cases where prevention or treatments are not yet available. Policy makers continue to grapple with these challenges.



The Future of Molecular Diagnostics

Conclusion

This report has only touched the surface of molecular diagnostics today. The power of the science, clear links to clinical outcomes, and the technological advances that are allowing molecular diagnostics to go from a translational research tool to mainstream clinical applications are impressive. We also are witnessing the transition of these technologies to settings outside the lab, where their impact may be even greater.

DxInsights and AdvaMedDx will publish additional reports that will continue to tell this story. These reports will include exploration of next generation sequencing technologies, health IT and treatment of complex algorithms in diagnostics, companion diagnostics, and global diagnostics needs.

Acknowledgments

Introduction to Molecular Diagnostics:
The Essentials of Diagnostics Series

DxInsights is a non-profit organization formed to elevate the understanding of diagnostics in the face of rising health care costs. Created by industry veterans involved in all facets of diagnostics including development, commercialization, regulatory, reimbursement and communications, DxInsights is focused on expanding the role diagnostics play in the future of health care and personalized medicine.

The goal of DxInsights is to conduct educational programs to guide industry colleagues, policy makers, payers and health care advocates to greater appreciation of the power and value of diagnostics and their direct impact on improving patient outcomes and reducing costs.

AdvaMedDx

The world's leading diagnostics manufacturers established AdvaMedDx to advocate for the power of medical diagnostic tests to promote wellness, improve patient outcomes, and advance public health in the United States and abroad.

AdvaMedDx represents one of the most dynamic and innovative sectors in the health care system. Diagnostics influence as much as 70% of health care decision making. These tests—performed in laboratories, at the hospital bedside, in doctor's offices, in medical clinics, and in the home—facilitate evidence-based medicine, improve quality of care, promote wellness, enable early detection of disease and often reduce health care costs.

For more information, news, and educational materials relating to this report or other diagnostic topics, please visit our web sites.

www.dxinsights.org

advameddx.org

This report was written by the DxInsights and AdvaMedDx teams, guided by Kristin Pothier and Ray Woosley, DxInsights, Andrew Fish and Tharini Sathiamoorthy, AdvaMedDx, and a team of industry and institutional reviewers.

Glossary of Common Molecular Diagnostic Terms

Allele
An allele is one of two or more forms of the DNA sequence of a particular gene. Different DNA sequences (alleles) can result in different traits, such as color of skin, hair or eyes. If both alleles are the same, the person is termed a homozygote. If the alleles are different, the person is a heterozygote.

Amplification
Amplification of DNA is a process in which the polymerase chain reaction (PCR) is repeated for a number of cycles or rapidly changing temperatures to exponentially increase the number of copies of a specific target region of a gene.

Biomarker
A biological property or substance(s) that is a sign of a normal or abnormal process, or of a condition or disease. A biomarker is used to determine how patients respond to treatments.

Diagnostics
The use of clinical tests to inform clinical decision making. The area includes both tests conducted on specimens from the body (i.e., in vitro diagnostics) and imaging tests (e.g., in vivo diagnostics), for the purpose of disease prediction, screening, diagnosis, treatment selection, prognosis and monitoring.

DNA
DNA is the acronym for deoxyribonucleic acid, the code used within cells to form proteins. In cells, DNA usually exists as two long intertwined chains twisted into a double helix and joined by hydrogen bonds between the complementary bases adenine and thymine or cytosine and guanine. The chains are composed on nucleotides (a combination of any one of the nucleic acid sequence of nucleotides in DNA determines individual hereditary characteristics.

Esoteric Test
The analysis of 'rare' substances or molecules that are not performed in a routine clinical lab.

Gene
A hereditary unit consisting of a sequence of DNA that occupies a specific location on a chromosome and determines a particular characteristic in an organism. Genes undergo mutation when their DNA sequence changes during cell division.

In Vitro Diagnostics (IVD)
In vitro diagnostic products are those reagents, instruments, and systems intended for use in diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae. Such products are intended for use in the collection, preparation, and examination of specimens taken from the human body.

Microarray
A microarray is a multiplex lab-on-a-chip. It is a 2D array on a solid substrate (usually a glass slide or silicon thin-film cell) that assays large amounts of biological material using high-throughput screening methods.

Molecular Diagnostics
Diagnostic tests that identify a disease, predisposition for a disease, or progress in treating a disease by detecting specific molecules such as DNA, antibodies, and proteins.

Mutation
In molecular biology and genetics, mutations are changes in a genomic sequence: the DNA sequence of a cell's genome or the DNA or RNA sequence of a virus. Mutations are caused by radiation, viruses and mutagenic chemicals, as well as errors that occur during DNA replication. Mutation can result in several different types of change in DNA sequences; these can have either no effect, alter the product of a gene, or prevent the gene from functioning properly or completely. Due to the damaging effects that mutations can have on genes, organisms have mechanisms such as DNA repair to remove mutations.

Glossary of Common Molecular Diagnostic Terms

Nucleic Acids
Nucleic acids are any of various complex organic acids (e.g., DNA or RNA) that are composed of nucleotide chains.

Nucleosides
Any of a class of organic compounds, including structural subunits of nucleic acids. Each consists of a molecule of a five-carbon sugar (ribose in RNA, deoxyribose in DNA) and a nitrogen-containing base, either a purine or a pyrimidine. The base uracil occurs in RNA, thymine in DNA, and adenine, guanine, and cytosine in both, as part of the nucleosides uridine, deoxythymidine, adenosine ordeoxyadenosine, guanosine or deoxyguanosine, and cytidine or deoxycytidine. Nucleosides usually have a phosphate group attached, forming nucleotides.

Nucleotides
Nucleotides are any of several compounds that consist of a ribose or deoxyribose sugar joined to a purine or pyrimidine base (adenosine, cytosine, thymidine, guanine or uracil) and to a phosphate group and that are the basic structural units of nucleic acids (as RNA and DNA).

Polymerase Chain Reaction (PCR)
A technique for amplifying DNA sequences in vitro by separating the DNA into two strands and incubating it with oligonucleotide primers and DNA polymerase. It can amplify a specific sequence of DNA by as many as one billion times and is important in biotechnology, forensics, medicine, and genetic research.

Polymorphism
The existence of a gene in several allelic forms of >1% frequency; also: a variation in a specific DNA sequence.

Primer
A segment of DNA or RNA that is complementary to a given DNA sequence and that is needed to initiate replication by DNA polymerase.

RNA
RNA is an acronym for ribonucleic acid. RNA is chain of alternating phosphate and ribose units with the bases adenine, guanine, cytosine, and uracil bonded to the ribose. RNA molecules are usually single stranded and involved in protein synthesis.

Sequencing
The technique used to map out the sequence of the nucleotides that comprise a strand of DNA.

Thermocycler
is a laboratory instrument used to amplify segments of DNA using PCR. The instrument has a thermal block which holds amplification tubes for each reaction. The thermocycler raises and lowers the temperature of the block in programmed steps to perform the reaction.