Chapter 5

Nucleic Acids — The Use of Nucleic Acid Testing in Molecular Diagnostics

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Additional information is available at the end of the chapter

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Abstract

In 1989 Roche entered into an agreement with Cetus to develop diagnostic applications for the novel technique polymerase chain reaction (PCR). A new area of molecular diagnostics began and genes and pathogen genomes have been used to diagnose disease since that point. Automated laboratory platforms were created to facilitate the workflow and allow for accurate and precise processing of patient blood samples in a highly streamlined manner. In this chapter the use of nucleic acids in molecular diagnostics will be described and their application to important human diseases. Examples are discussed with respect to which nucleic acid marker has provided strong clinical utility and impact to healthcare.

Keywords: Real Time-PCR, molecular diagnostic, HIV-1, human genetic testing

1. Introduction

In this chapter, the use of nucleic acids in molecular diagnostic testing will be described. Detailed disease area examples will be discussed to illustrate technical capabilities as well as the medical relevance of such testing.

Polymerase chain reaction (PCR) was invented by Kary B. Mullis in the 1980s. Fundamentally, PCR is a cyclic process designed to specifically replicate (amplify) nucleic acid sequences from as little as one to a few strands of DNA. The target DNA is heated to separate double-stranded DNA sequences; short oligonucleotide "primers" that define the portion of the genome to be replicated bind to the target DNA. The primers are extended by a DNA polymerase making a copy of the target DNA. After multiple cycles in which the concentration of the replicated target DNA increases exponentially, the amplified product (amplicon) can be visualized by gel electrophoresis or measured by detection of labeled PCR product (amplicon) by incubation

with additional reagents to produce color or by fluorescent probe detection. This technique improved the ability to diagnose a number of diseases by enabling identification of many human pathogens that had previously been difficult to detect due to their low concentration in the sample. With the addition of a reverse transcription step to the original PCR process, RNA could be converted to cDNA and then replicated with the PCR process. Thus, the utility of the technique was broadened to detect RNA viruses and eukaryotic mRNA.

A key technical improvement was introduced by Higuchi et al.[4, 5] who developed real-time polymerase chain reaction (RT-PCR), which follows the kinetics of the PCR and detects PCR products during the process of amplification (Figure 1). With RT- PCR, accurate and reproducible quantitation of pathogen concentration could be incorporated into the amplification process. RT-PCR is used to monitor a pathogen's kinetic replication processes over time, and measurement of viral load is now widely employed to monitor the success of treatment of viral and other diseases.

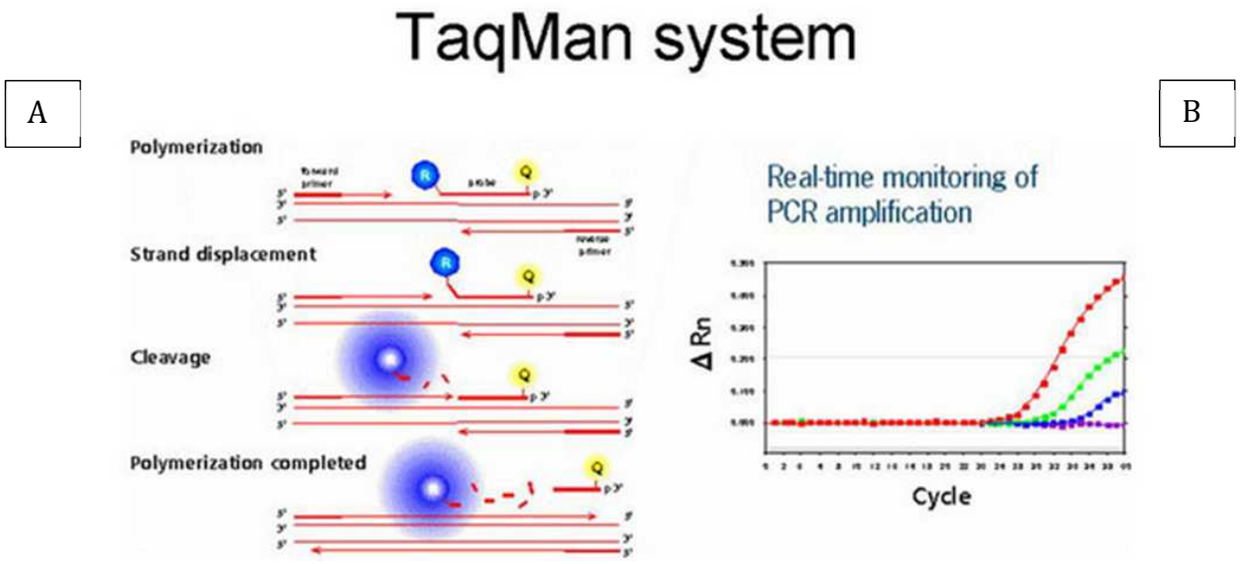

Figure 1. Principles of Real-Time PCR. (A) During the polymerization step, the template is amplified by primers supplied in the reaction mix. The amplicon allows for annealing of sequence-specific, labeled probes. As a new strand is synthesized, the probes will be displaced, the label cleaved off, and a fluorescent signal proportional to the amount of the cleaved probe is generated. (B) Fluorescence is measured and recorded at each cycle of PCR. Cycle threshold (Ct) is defined as the fractional PCR cycle number in which the sample fluorescence signal reaches a level above an assigned fluorescence threshold. The Ct value indicates the beginning of the exponential amplification of the template DNA or RNA and is proportional to the concentration of the sample.[6]

Clinical microbiology was one of the first fields to adopt PCR and, later, RT-PCR, due to the sensitivity and specificity of the technique for detecting nucleic acids of pathogenic microorganisms.

Perkin-Elmer developed the first thermal cycler instrument in December 1985. The first commercial *in vitro* PCR diagnostic products were created when the California company, Cetus, entered into a partnership with Kodak in February 1986.[7] The first reagent kit, the "Gene-Amp PCR reagent kit" and the thermal cycler were commercially available in November 1987.[8]

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In January 1989, it was announced that Roche had entered into an agreement with Cetus to develop diagnostic applications for PCR. A new area of molecular diagnostics began using PCR to detect genes and pathogen genomes to diagnose diseases since that time.[7]

At Cetus, it was decided in the late 1980s that the forensic applications of PCR represented a stand-alone business that could be operated in-house, and, therefore, the applications were not sold to a partner.[7] In 1990, the first forensic PCR kit, developed by Cetus, was sold by Perkin-Elmer and became useful for identification of individual humans. The nucleic acid, DNA, became a mainstay of the justice system in 1997 when the FBI announced the selection of 13 short tandem repeat (STR) DNA loci to constitute the core of a national database—Combined DNA Index System (CODIS). By the time a review was published in 2006,[9] 5 million profiles of individuals existed in CODIS. By 2003, almost 1 million samples were being processed annually using core STR loci as part of parentage testing.[10]

Over the past decades, RT-PCR technology has continued to develop, optimize, and expand in the clinical laboratory for the identification, detection, and quantitation of a variety of pathogen uses. Automated instrument platforms were created to facilitate the workflow and allow for accurate and precise processing of patient samples in a highly automated manner.

2. Nucleic acid detection in molecular diagnostics

For molecular diagnostic purposes, since each microbe has a unique complement of DNA (or RNA, for many viral pathogens), nucleic acids are the ideal molecular fingerprint aiding identification. Particularly useful are RT-PCR and PCR, with their enzyme-driven processes for amplifying RNA/DNA *in vitro*, to analyze levels of microbial DNA in clinical samples for which other detection methods require higher concentrations, or are too time-consuming or cumbersome to detect.

2.1. The importance of nucleic acid measurement for HIV-1

With the emergence of the global HIV epidemic in the 1980s, it became evident that following the viral kinetics in infected subjects can aid significantly in understanding the progression of HIV-1 infection to stage 3, i.e., the disease of AIDS. Before viral load tests, many researchers believed that HIV-1 infection underwent dormant periods. Viral load tests showed that HIV-1 replication in the human body is a continual and gradually progressive process and that the viral replication is always active.[11] A typical pattern of HIV-1 infection is shown in Figure 2.

With the development of the first antiretroviral (ARV) agents (such as AZT, zidovudine) and later, the establishment of highly active antiretroviral therapy (HAART), understanding the kinetics of suppression of viral replication and detection of antiviral resistance became a major focal point in guiding and caring for patients. Viral load monitoring was the first direct approach to personalized healthcare, determining the activity of ARV medicines in an individual patient at specific time points.

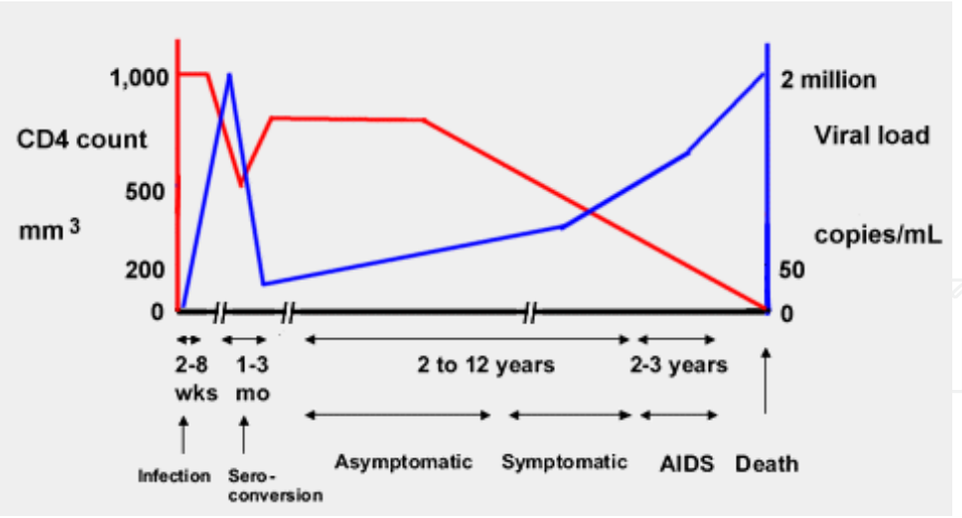

Figure 2. Viral Kinetics of HIV-1 Infection. After HIV-1 infection, during the acute disease phase, viral load is high, followed by a strong CD4 cell decline. After seroconversion and establishment of chronic infection, the viral load reaches a viral set point phase (at approximately 14 weeks following infection) from which it continues to rise as the CD4 cell count declines over several years. A CD4 cell count of fewer than 200 cells/mm^3 is one of the qualifications for a diagnosis of stage 3 infection (AIDS). Source: http://i-base.info/ttfa/section-2/214-how-cd4-and-viral-load-are-related/[12]

Suppressing the HIV-1 viral load to undetectable levels (<50 copies per mL) is the primary goal of HAART.[13] This level of suppression should be achieved by 24 weeks after starting combination therapy. HIV-1 viral load is the most important predictor of response to treatment with HAART.[15] Failure of HAART to adequately suppress viral load is termed virologic failure. Levels of HIV-1 RNA higher than 200 copies per mL are considered virologic failure, and should prompt further testing for potential viral resistance.

In 1992, to aid in this therapeutic approach the first commercially available PCR-based diagnostics were marketed. The AMPLICOR CT (*Chlamydia trachomatis*) Test and the AMPLICOR HIV-1 MONITOR Test were the first PCR-based molecular diagnostic tests. During the first half of the 1990s, the sensitivity of these early commercial products was moderate. By 1996 to 1997, through technical improvements, the next generation of tests could detect and measure viral loads as low as 400 or 500 copies/mL. Since 1998, most tests used routinely in clinical practice accurately detect and measure HIV-1 RNA as low as 40 or 50 copies/mL.[18] For academic research purposes, several groups have described an ultrasensitive or single copy assay that can detect 5 copies/mL or even 1 copy/mL.[19]

In addition to RT-PCR, a range of other nucleic acid-based techniques are also employed to measure HIV-1 RNA viral load, such as branched DNA (bDNA) assay[20] and nucleic acid sequence based amplification (NASBA).[21] NASBA was developed in 1991 by J. Compton, who defined it as "a primer-dependent technology that can be used for the continuous amplification of nucleic acids in a single mixture at one temperature."[22]

These techniques were also employed to study the relative effectiveness of ARV drugs in clinical trials during the very active HIV drug discovery decades from 1990 to 2010.[23]

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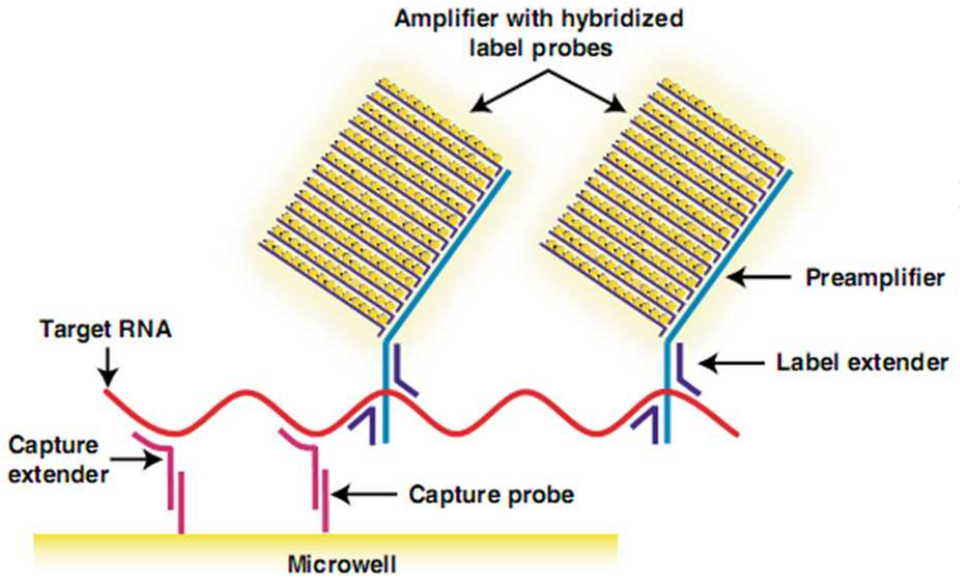
(A) bDNA



(B) NASBA

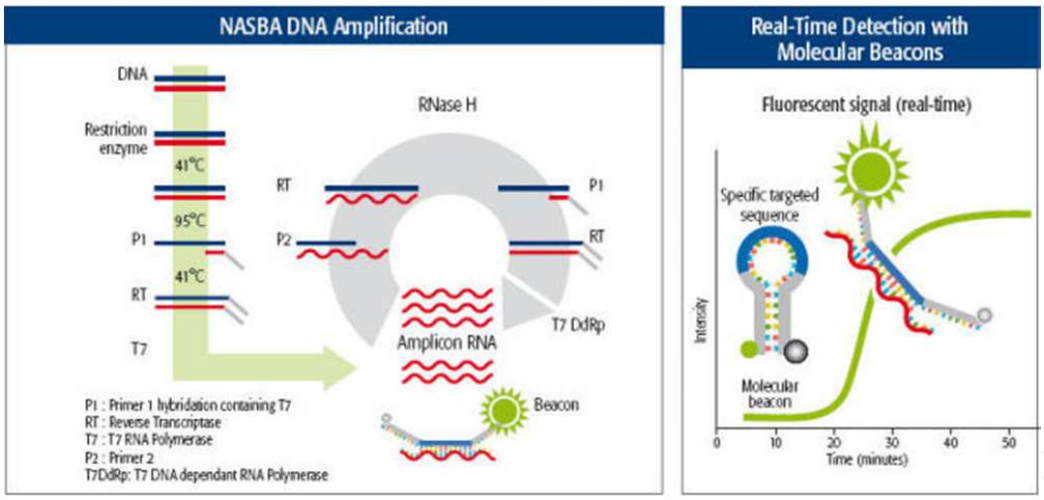


Figure 3. Principle of bDNA and NASBA. (A) Target RNA is captured with a bifunctional Capture Extender oligonucleotide probe that hybridizes to the target molecule and a Capture Probe that is covalently attached to a substrate (e.g., a microtitre plate well or a bead). A Signal Amplification complex (Preamplifier and Amplifier with labeled probes) containing a number of alkaline phosphatase enzymes is then hybridized to the target molecule via a Label Extender probe. Source: http://www.diacarta.com/article.php?id=38[24] (B) NASBA works as follows: An RNA template is added to the reaction mixture and reverse transcriptase synthesizes the opposite, complementary DNA strand. RNAse H destroys the RNA template from the DNA–RNA complex (RNAse H only destroys RNA in RNA–DNA hybrids, but not single-stranded RNA). A second primer attaches to the 5' end of the DNA strand. Reverse transcriptase again synthesizes another DNA strand from the attached primer, resulting in double-stranded DNA. T7 RNA polymerase continuously produces complementary RNA strands off this template which results in amplification. Finally, a molecular beacon is employed to detect the amplified product and allow for quantitation. Source: http://www.biomerieux.com.co/servlet/srt/bio/colombia/dynPage?open=CLM_CLN_PRD&doc=CLM_CLN_PRD_G_PRD_CLN_87&pubparams.sform=3&lang=es_co[25]

Various nucleic acid detection techniques, discussed above, are employed for the detection and quantitation of HIV-1 infection. Molecular diagnostics may carry, in general, a larger cost burden than other laboratory techniques that detect pathogens via shed surface proteins or antibodies in human serum. However, the speed, specificity, and sensitivity of molecular testing offers a number of advantages over the more "traditional" methods that it has replaced, such as culture, which is slow and labor-intensive, or hybridization or similar techniques that are often imprecise, insensitive, or for which the interpretation of results is often subjective. Molecular methods also offer advantages over measuring antigens, such as p24, or disease markers, such as CD4 cells, in people infected with HIV-1. Early monitoring of the status of patients with HIV-1 infection used the CD4 cell count to determine progression of disease, and many resource-limited settings still employ this technique today. CD4 cells are the white blood T-cells that are specifically targeted by HIV due to their surface receptor repertoire and depleted as infection progresses. The CD4 cell count provides a measure of the immune function of the human host and is a late marker of disease progression. The measurement is used in establishing thresholds for the initiation and discontinuation of opportunistic infection (OI) prophylaxis and in assessing the urgency to initiate HAART. It is recommended that ARV therapy be initiated when the CD4 cell count falls below 200–350 cells/mm^3, depending on the availability of ARV medicines in a given country.

Measurement of CD4 cells using the current technology is imprecise. Since certain standard-of-care recommendations, such as initiation of prophylaxis against *Pneumocystis carinii* pneumonia (an OI common in HIV-1 patients), have been made, treatment may be based on a single CD4 cell count, and CD4 measurement error may have important clinical consequences. Often the use of confirmatory tests is recommended and both tests need to be below a certain threshold limit.[27] Therefore, additional cost is incurred by the confirmatory testing, and the advantage of using the more inexpensive CD4 cell count test is lost.

After initiation of ARV therapy, due to suppression of the HIV-1 viral load, the immune system is allowed to recover and the CD4 cell count increases. For most patients on therapy, an adequate response is defined as an increase in CD4 count in the range of 50–150 cells/mm^3 during the first year of HAART, generally with an accelerated response in the first 3 months of treatment. The CD4 count response to HAART varies widely, but a poor CD4 response in a patient with viral suppression is rarely an indication for modifying an ARV regimen. In patients with consistently suppressed viral loads who have already experienced HAART-related immune reconstitution, the CD4 count provides only limited information.[28]

A second biomarker used in HIV-1 laboratory testing is the viral core protein p24. This biomarker can be measured in the patient's blood in early acute infection, often before antibodies to the viral onslaught are detectable. A negative result for the antigen does not rule out infection, because the test lacks exquisite sensitivity; i.e., the test should not be used to verify noninfection. Antigen detection signals infection, however, and positive results in seronegative individuals can be an effective, although not cost-effective, means to identify early infection. The p24 antigen test can be of value in blood screening, for identification of acute infection, for monitoring infection, and to assist in the diagnosis of infection in the newborn. It has been used for detecting early infection in rape cases, for identifying infection

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after occupational exposure, and for assisting in the resolution of indeterminate Western blot results.[29]

As both CD4 cell count and p24 have caveats briefly discussed above, HIV-1 RNA viral load analysis by nucleic acid testing has, in many clinical situations, replaced less predictive methods of measurement of these biomarkers.

2.2. Other therapeutic areas

2.2.1. Microbiology and infectious diseases

Real-time PCR revolutionized the means by which clinical laboratories identify human pathogens. It is estimated that <1% of bacteria present on earth have been described using cultivation technology.[30] Additionally, various pathogens, particularly mycobacteria and fungi, require prolonged periods of cultivation, necessitating administration of empiric antimicrobial therapy while a laboratory result is awaited. Due to the limitations of cultivation technology, PCR amplification and sequencing-based methods are able to also reveal novel microbes associated with human diseases. Hence, cultivation-independent methods offer a potential for rapid diagnosis, thus preventing antibiotic selection pressure and emergence of resistant pathogen infections. Additionally, molecular testing is able to identify hazardous microbes without risk to laboratory staff as well as speed isolation of a given patient harboring highly infectious pathogens into a quarantine setting.

As opposed to monitoring during care of chronic viral infections, such as HIV-1, HBV, and HCV, most tests in the microbiology diagnostic assay repertoire are qualitative, aimed at the detection of the pathogens, which then warrant follow-up evaluation.

Several reviews of real-time PCR in clinical microbiology have been published; among others, the review by Espy et al. in 2006 is a comprehensive guide. Important pathogens diagnosed with molecular testing at the time of publication[32] of this chapter were agents for disease areas such as:

- Respiratory infections, such as adenovirus, Mycoplasma pneumoniae, Mycobacterium tuberculosis, Legionella spp, and Streptococcus pneumonia
- Genitourinary/sexually transmitted infections with PCR assays for C. trichomatis, Neisseria gonorrhoeae, Mycoplasma genitalium, and human papillomavirus
- Central nervous system infections dominated by herpes simplex, varicella zoster, and West Nile Virus (WNV)
- Gastrointestinal infections with, most notably, Clostridium difficile

In recent years, infectious disease surveillance and monitoring of antibiotic resistance has also been added to PCR-based molecular diagnostic tests, such as detection of gram-negative bacilli and vancomycin-resistant enterococcus species.

Finally, the host of viral pathogens causing human disease are generally identified, quantitated, and managed via PCR-based laboratory tests. Important examples are diagnosis and

management of hepatitis B and C, herpes virus family infections, and influenza epidemic outbreaks.

2.2.2. Blood screening

Annually, millions of people worldwide receive blood transfusions or blood-derived products. Around the world, more than 92 million blood donations are collected every year.[33] From these, a single whole-blood donation can be transfused in up to three people, and blood-derived products from a single donation may be given to hundreds of patients.[34, 35] Although testing and policy decisions have combined to make blood supplies in many countries among the safest in the world, there still exists some risk of transfusion-transmitted infection (TTI) with blood-borne diseases (e.g., HIV, hepatitis, WNV). Laboratory screening of donated blood and blood products for infectious diseases is a key safety measure in protecting patients and preventing the spread of serious diseases.

Nucleic acid testing (NAT) by PCR- or transcription-mediated amplification (TMA) technology detects the presence of viral infection by directly testing for viral nucleic acids and can be used to screen whole blood and plasma samples. Commonly used NAT assays detect HIV-1 RNA, HCV RNA, HBV DNA, and WNV RNA.[38]

NAT technology has revolutionized the ability of blood centers to efficiently test for and reduce infusions of potentially infectious blood units while continuing to ensure on-time availability of blood and blood products for patients. The global trend toward adopting this technology clearly demonstrates its effectiveness for increasing the safety of blood supplies.

2.2.3. Human genetics—Testing via nucleic acid markers

Besides the exploration of human pathogen diagnostics, molecular testing has been employed to identify a myriad of human host markers predominately via DNA found in any human cell.

2.2.3.1. Prenatal diagnosis

Prenatal diagnosis employs a variety of techniques to determine the health and condition of an unborn fetus. There are three purposes of prenatal diagnosis: (1) to enable timely medical or surgical treatment of a condition before or after birth, (2) to give the parents the chance to abort a fetus with the diagnosed condition, and (3) to give parents the chance to prepare psychologically, socially, financially, and medically for a baby with a health problem or disability or for the likelihood of a stillbirth.

Congenital anomalies account for 276,000 perinatal deaths by pregnancy Week 4 annually on a global basis. The aim of prenatal screening is to detect birth defects, such as neural tube defects; chromosome abnormalities (e.g., Down Syndrome, fragile X syndrome); and genetic disorders and other conditions (e.g., spina bifida, cleft palate, Tay Sachs disease, sickle cell anemia, thalassemia, cystic fibrosis, and muscular dystrophy). Screening can also be used for prenatal sex discernment.

There is a variety of noninvasive and invasive techniques available for prenatal diagnosis. Each should be applied only during specific time periods of a pregnancy for greatest utility.

Traditionally, amniocentesis, performed at pregnancy Weeks 14–20, was employed to sample the amniotic fluid, which contains fetal cells, for analysis of chromosomal defects. Risks with amniocentesis are uncommon, but include fetal loss. The increased risk for fetal mortality following amniocentesis is about 0.5% above what would normally be expected. Collected embryonic cells from the amniotic sac need to be cultured for the chromosomal analysis. This process is cumbersome, carried out in specialized laboratories only, and requires a time period of 1–2 weeks, including transport of the sample.

Similarly, chorionic villi sampling can provide information about the fetus' health and development status as early as at 10 weeks of pregnancy. Miscarriage rates are higher in this procedure compared to amniocentesis, up to 1.9%. Test results are obtained within 2 weeks and require specialized laboratories and culturing techniques.

In recent years, analysis of cell-free DNA shed from fetal cells in the maternal blood has become a molecular technique to investigate congenital defects as early as pregnancy Weeks 9–10. High-throughput shotgun sequencing of the plasma of pregnant women results in obtaining about 5 million sequence tags per patient sample. Using this technology, in 2008, Fan et al. were able to identify aneuploid pregnancies, with trisomy detected at gestational ages as early as ~10 weeks. Shotgun sequencing is carried out on a next-generation sequencing platform such as Illumina. In 2010, Chiu et al. studied 753 pregnant females using a 2-plex massively parallel maternal plasma DNA sequencing, and trisomy was diagnosed with z-score greater than 3.[43] The test demonstrated 100% sensitivity, 97.9% specificity, positive predictive value of 96.6%, and negative predictive value of 100%.

The main advantages of these protocols are that they can be used earlier than the current prenatal testing protocols and, unlike current protocols, that there is no risk of spontaneous abortion. Noninvasive prenatal diagnosis (NIPD) has been implemented in the United Kingdom (UK) and parts of the United States (US).

2.2.3.2. Inherited diseases

Carrier screening, testing of parents in preparation for pregnancy, is used to identify genetic mutations that could cause serious inherited disorders. Some of the more common disorders for which screening is done are cystic fibrosis, sickle cell disease, thalassemia, and Tay-Sachs disease. These disorders are recessive, which means that a person must inherit a defective gene from each parent to have the disease. If both parents are carriers of a disorder, the child will have a one-in-four chance of inheriting one defective gene from each of the parents and having the disorder. This type of testing is offered to individuals who have a family history of a genetic disorder and to individuals in certain ethnic groups with an increased risk of specific genetic conditions. For the testing procedure, venous blood is collected and sent to specialized laboratories. There, the DNA contained in the human blood cells is amplified via PCR and, for example, a next-generation sequencing platform is utilized to investigate the genotype of a set of genes in a cost-efficient manner.

Newborn screening is used just after birth to identify genetic disorders that can be treated early in life. Early detection, diagnosis, and intervention can prevent death or disability and enable children to reach their full potential. Each year, millions of babies in the US are routinely screened, using molecular tests performed on a few drops of blood obtained from their heels, for certain genetic, endocrine, and metabolic disorders, and are also tested for hearing loss prior to discharge from a hospital or birthing center. All states currently test infants for phenylketonuria (a genetic disorder that, if left untreated, causes intellectual disability) and congenital hypothyroidism (a disorder of the thyroid gland).

The expansion of the screening panel to approximately 30 heritable metabolic conditions occurred from 1997 to 2007 with the introduction of tandem mass spectrometry (MS/MS), a technology that detects multiple disease biomarkers simultaneously in a single specimen. This technique employs the screening of blood spots for inborn errors of metabolism by electrospray MS/MS with a microplate batch process and a computer algorithm for automated flagging of abnormal profiles. More recently, other markers, based on nucleic acid analysis of the newborn genetic makeup,[48] such as sickle cell disease, alpha-1-antitrypsin deficiency, and Factor V Leiden, have been added.

2.2.3.3. Cancer markers

Cervical cancer is the 7th most common cause of cancer death in Europe for females, and the 15th most common cause of cancer death overall. According to currently available US Centers for Disease Control (CDC) Fast Stats [49] cervical cancer mortality in the US in 2010 was ~4,000 or ~2.5 deaths per 100,000 females.

The global statistics provided by Cancer Research UK are far more saddening. Worldwide, there were more than ~275,000 deaths from cervical cancer in 2010 that accounted for ~10% of female cancer deaths.

The Papanicolaou test—aka Pap test, Pap smear, cervical smear, or smear test—was historically the method of cervical screening used to detect potentially precancerous and cancerous cells in the endocervical canal of the female reproductive system. Atypical findings were followed with more sensitive diagnostic procedures, and, if warranted, interventions that aimed to prevent progression to cervical cancer.

In March 2014, the FDA's Medical Devices Advisory Committee Microbiology Panel voted unanimously to approve the cobas® 4800 HPV Test (Roche Molecular Systems) and recommended that this real-time PCR HPV test replace the Pap smear as the first-line standard of care for cancer screening, another use of nucleic acid testing in molecular diagnostics.

Another wide-ranging use of molecular tests using PCR can be found in the disease area of colorectal cancers where tumor nucleic acids are analyzed for the presence of mutations or other markers. Historically, all colorectal cancers (CRCs) have been considered a single disease entity sharing the same cause, clinical characteristics, and treatment outcomes. However, through analysis of precursor lesions and hereditary forms of the disease, it has now become clear that CRC is a complex and heterogeneous disorder. Although microsatellite instability (MSI) testing has been used for more than a decade for identifying patients with Lynch

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syndrome, with the recent growth in personalized cancer care, other molecular tests to identify the genetic makeup of individual cancers have become increasingly more important in making therapeutic decisions. Novel medicines in oncology and relevant biomarker tests are now often developed side-by-side. Current indications for standard-of-care molecular testing in colorectal carcinomas include identifying hereditary cancer syndromes, such as Lynch syndrome (also known as hereditary nonpolyposis colorectal cancer [HNPCC]), and testing for *KRAS* mutational status as a predictor of response to antiepidermal growth factor receptor (EGFR) agents such as cetuximab. In the case of Lynch syndrome, multiple mononucleotide markers are detected via a fluorescent multiplex PCR-based method. A tumor tissue specimen (with tumor cellularity of >20%) and normal tissue specimen are amplified using PCR for 5–7 microsatellite markers. Patterns of normal and tumor genotypes are compared for each marker and scored as MSI-High, MSI-Low, or MS-Stable. Analysis for somatic mutations in the V600E hot spot in the BRAF gene may be indicated for tumors that are scored as MSI-High or show loss of MLH1 expression, because this mutation has been found in sporadic MSI-High tumors but not in HNPCC-associated cancer. *KRAS* mutations have been convincingly associated in randomized clinical trials with poor response to cetuximab and panitumumab. Activating mutations in *KRAS* serve to isolate this signaling pathway from the effects of EGFR and render EGFR inhibition ineffective. Recent advances have shown that only tumors with wild-type *KRAS* show significant response to these agents. Accumulated data from both randomized and nonrandomized studies, reviewed by Jimeno et al., suggest that patients with CRCs whose tumors show *KRAS* mutations should not receive EGFR-targeting monocloncal antibody therapy. This led to the so-called codiagnostic assays with guidance language in both the test intended use information and drug package insert detailing use of the molecular test results for physicians and patients. This approach of diagnostic testing prior to prescription of costly and not always easy-to-tolerate medicines will dominate personalized healthcare in the future.

Currently, most assays can be performed on small quantities of formalin-fixed paraffin-embedded–derived tumor DNA. The pathologist must carefully select the tumor block to minimize dilution of tumor DNA by contaminating normal cells, such as fibroblasts, endothelial cells, and inflammatory cells; a target of at least 10% tumor cells is recommended for most assays.

Cancer research continues to focus on new molecular markers.[55] The integration of molecular markers into existing histomorphologic classifications in surgical pathology has already provided additional stratification for a more accurate prognosis. Furthermore, a molecular definition of cancer may often guide therapy and allow the monitoring of residual disease.

3. Conclusion and a future outlook

The introduction of nucleic acid testing into clinical laboratories has vastly improved detection of infections. Chronic viral infection can be treated with tests at hand that are adequate to inform the physician if the patient is responding, developing resistance, or being cured. The safety of the blood supply was dramatically improved on a global basis with the introduction of nucleic acid testing for blood-borne pathogens. Expectant parents can be informed of the

genetic risks of a pregnancy and the inherited diseases for which a developing fetus or a newborn may be treated. Finally, today, patients diagnosed with cancer can experience a much more tailored approach to therapy, maximizing success and efficiency and minimizing costs to both themselves and the healthcare system.

The past decade has seen the number of commercial molecular tests used in practice increase fivefold. In 2013, 60% of the molecular diagnostics tests were sold by five companies: Roche, Becton Dickenson, Abbott, Hologic, and Qiagen.[56] However, in recent years the number of companies developing molecular tests has grown remarkably. Roughly, 350 companies are now active in development of molecular diagnostics,[56] highlighting the utility and importance of nucleic acid testing in healthcare today.

As molecular testing becomes more widely available and applicable to healthcare globally, it is not surprising that the next-wave nucleic acid testing will penetrate the markets in emerging and developing countries. For example, ARV regimens are becoming more widely available, including in sub-Saharan Africa to manage the large numbers of HIV-infected individuals, and state-of-the-art viral load testing will need to accompany the expansion of these regimens. It is a challenge to the manufacturers of nucleic acid tests to adapt technologies and platforms to resource-limited settings. The future of molecular testing may involve reduction in time to test result as well as reduction in assay and instrument complexity and number and training expertise of staff required to perform such assays.

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