Background Information

Roche Diagnostics

PCR – PIONEERED BY THE INNOVATORS AT ROCHE DIAGNOSTICS

Polymerase chain reaction (PCR) is the basic technology for the new AMPLICOR Human Papillomavirus (HPV) test kit with CE marking ("Conformité Européenne" for the European Union). It is the first PCR-based reagent for the detection of HPV and identifies all 13 high-risk genotypes of HPV, which is the leading cause of cervical cancer affecting more than 500,000 women worldwide every year.

PCR is a Nobel-prize-winning technology used to amplify, or copy, specific sequences of genetic material (called deoxyribonucleic acid, or DNA). Using this technology, scientists can produce large enough quantities of DNA to be able to examine it in detail for a variety of different purposes, including:

- Identifying viruses
- Predicting how well an individual will respond to a medication
- Diagnosing specific genetic conditions or illnesses
- Establishing maternity, paternity, or other blood relationships.

PCR's impact on diagnostics and healthcare

The immediate and far-reaching implications of PCR cannot be overstated. Using PCR, scientists can take a specimen containing a minute amount of genetic material, copy a selected region from it over and over, and – within hours – generate a sample sufficient to perform a variety of tests.

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PCR is very versatile. Many types of sample (blood, skin cells, saliva, and hair, to name a few) can be analyzed for nucleic acids. The main criterion for any sample used for PCR is that it must contain the DNA strand encompassing the region to be amplified.

One of the first diagnostic applications of PCR was for the detection of HIV-1, the virus that causes AIDS. PCR also helps doctors determine exactly how much of the HIV-1 virus is present in the blood of those who carry this virus. This can help them make vital decisions about treatment and monitor the patient's response to that treatment. The new Amplicor HPV test marks the latest development in this ground-breaking technology by Roche Diagnostics.

Development of PCR

First described in the journal *Science* in 1985, PCR has become one of the most widely used techniques in molecular biology, and for good reason: from the daily practicalities of medical diagnosis to the courts of law, PCR takes the analysis of tiny amounts of genetic material to a new level of precision and reliability.

Because it is far simpler and less expensive than previous techniques for analyzing DNA (Southern Blot, for example), PCR has "democratized" genetic research, putting it within reach of all biologists – even those with no previous training in molecular biology.

The PCR process

The following is a basic overview of the PCR process:

- DNA samples extracted from the specimen are **denatured**, or split into two separate strands. (DNA naturally forms a two-stranded, "double-helix" shape.)
- Next comes **annealing**, where primers – short, synthetic sequences of single-stranded DNA (such as primers in Roche Diagnostics' Amplicor Test) – are used to mark off the selected segment of DNA sequence to be studied.

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- Once the primers anneal to the DNA sequences, the temperature is raised to approximately 72°C. The enzyme 'Taq DNA polymerase' is used to copy, or replicate, the DNA strands in a process called **extension**. This process takes place in a thermal cycler, an instrument that automatically controls and alternates the temperatures for programmed periods of time for the appropriate number of PCR cycles (usually between 30 and 40 cycles). Each cycle doubles the amount of replicated DNA.

Diagnosing elusive diseases

PCR very quickly became an essential tool for improving human health in two critical areas – the diagnosis of infectious disease organisms and the detection of genetic variations and mutations. Because PCR can amplify minute amounts of DNA (one cell is enough), physicians and researchers can analyze a single sperm cell, or track down the source of a viral infection.

More than 60 PCR protocols for identifying different diseases have been described to date. Here are just a few infectious organisms that can now be detected using PCR:

- HIV-1
- Chlamydia
- Hepatitis C
- Hepatitis B
- Neisseria gonorrhoeae
- Borrelia burgdorferi (the bacteria that cause Lyme disease)
- Helicobacter pylori (an organism that causes stomach ulcers)
- Mycobacterium tuberculosis
- Human Papillomavirus (HPV)
- Herpes Simplex Virus 1, 2
- Enteroviruses
- Cytomegalovirus
- SARS virus
- West Nile virus

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The Roche Diagnostics commitment

Since 1991, Roche Diagnostics has invested hundreds of millions of dollars in research and development to accelerate the development of PCR technology, with an emphasis on practical applications with direct benefits to healthcare.

The Roche Diagnostics commitment has included several million dollars in grants to academic institutions, government laboratories, and non-profit organizations for PCR-related research, as well as the continuing dedication of more than 250 Roche Diagnostics scientists and engineers devoted exclusively to the further development of the technology.

Roche Diagnostics retains a focus on six distinct areas – virology, blood screening, women's health, microbiology, oncology, and genomics. The company's automation technology platforms seek to enable the emergence of a brand-new diagnostics industry by making PCR analysis faster, less expensive, and more accessible, with broader-reaching applications.

The future of PCR technology

Twenty years ago, PCR began the process of revolutionizing healthcare by enabling understanding of the body and infectious agents at the molecular level. Today, Roche Diagnostics remains committed to pioneering new ways to use PCR not only to diagnose disease and monitor response to therapy, but to develop tests that help guide the physician's choice of therapy as well.

Roche Diagnostics is also pioneering advances in instrumentation for PCR testing, which is becoming smaller, more automated, and easier for a wider variety of laboratories to use. In essence, it is the Company's goal to help put molecular technology in virtually every laboratory. Roche Diagnostics' Cobas TaqMan Analyzer and Cobas TaqMan 48 Analyzer represent the Company's first clinical real-time PCR instruments that combine clinical and research features in one instrument. These instruments truly represent Roche Diagnostics' continued commitment to the evolution of PCR technology. By first using the Roche Diagnostics Cobas AmpliPrep Analyzer to prepare PCR specimens, and then employing the Cobas TaqMan 48 Analyzer, which automates the amplification, detection and quantitation of

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DNA or RNA, PCR analysis is fully automated. The initial menu for these new instruments is: PCR tests for HIV (human immunodeficiency virus), HBV (hepatitis B virus), and HCV (hepatitis C virus).

New uses are being found for PCR every day. Researchers at Roche Diagnostics and around the world use PCR to better understand and predict diseases – such as osteoporosis and heart disease – that we do not ordinarily consider genetic conditions. Someday, doctors may be able to use information gathered from this study of the body at the molecular level to prevent diseases before they occur, or even to correct specific genetic mutations responsible for diseases. This shows the revolutionary potential for new approaches to medicine set in motion by discoveries like PCR.

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