THE IMPORTANCE OF PCR IN THE LABORATORY

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Abstract: The core principle of PCR is the use of an enzyme called DNA polymerase to make a copy of a DNA strand. Normally DNA exists as a double strand, but the enzyme can only work on a single strand. Therefore it is first necessary to separate the strands of DNA. This is done by applying heat. The capacity of the technique called the polymerase chain reaction (PCR) to amplify many million-fold any known DNA fragment from a complex mixture in a short time has revolutionized all areas of the life sciences, making it one of the most widely used molecular techniques in use today. Both single strands of the original double-stranded DNA molecule are copied. Therefore the result is two double stranded molecules each identical to the original double-stranded DNA fragment.

Key words: PCR, differential diagnosis, pathogenesis, DNA, RNA, laboratory.

ВАЖНОСТЬ ПЦР В ЛАБОРАТОРИИ

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Аннотация: Основным принципом ПЦР является использование фермента, называемого ДНК-полимеразой, для создания копии цепочки ДНК.

Обычно ДНК состоит из двух цепей, но фермент может работать только с одной цепью. Поэтому сначала необходимо разделить нити ДНК. Это делается путем применения тепла. Способность метода, называемого полимеразной цепной реакцией (ПЦР), многократно усиливать любой известный фрагмент ДНК из сложной смеси за короткое время произвела революцию во всех областях наук о жизни, сделав его одним из наиболее широко используемых молекулярных методов на сегодняшний день. Копируются обе отдельные нити исходной двухцепочечной молекулы ДНК. Таким образом, в результате получаются две двухцепочечные молекулы, каждая из которых идентична исходному фрагменту двухцепочечной ДНК.

Ключевые слова: ПЦР, дифференциальная диагностика, патогенез, ДНК, РНК, лаборатория.

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Quite simply, PCR is a laboratory technique used to makes a huge number of copies of a piece of DNA. In other words it is a way of amplifying DNA or increasing the amount of a specific piece of DNA. The core principle of PCR is the use of an enzyme called DNA polymerase to make a copy of a DNA strand. Normally DNA exists as a double strand, but the enzyme can only work on a single strand. Therefore it is first necessary to separate the strands of DNA. This is done by applying heat. Applying heat to DNA denatures the double strand to single strands. In the first PCR step the double strand melts to single stranded DNA, a step known as denaturation. The enzyme can work on the single strand to make a copy. However, the enzyme needs a small region of double strand to get started. So it is necessary to add a short piece of single strand DNA, called the primer , that binds specifically to a particular place on the single strand molecule. This binding, or annealing, is achieved by cooling the PCR mixture again. This step in the PCR process is called annealing.

Introduction: Hepatitis B and C are serious viral diseases that affect millions of people worldwide, leading to liver inflammation and impaired liver function. Timely detection and confirmation of these viral infections are critical, as early diagnosis significantly increases the chances of successful treatment. Polymerase Chain Reaction (PCR) technologies represent a major breakthrough in this area, as they enable the detection of viral infections at the molecular level. In this article, the process of confirming hepatitis B and C infections using PCR technologies, the achievements, and existing limitations will be discussed in detail.

Theoretical Basis of PCR Technology: PCR technology is a molecular biology method used to amplify nucleic acids, allowing for significant replication of viral DNA or RNA extracted from a patient's blood or other biological samples. In the case of

hepatitis B and C infections, this technology is used to detect the viruses' genetic material and confirm their presence.

The PCR process consists of three main stages:

1. Denaturation: During this stage, the DNA or RNA strands in the sample are separated by heating.
2. Annealing: The temperature is lowered, and PCR primers bind to specific parts of the DNA.
3. Elongation: The DNA polymerase enzyme extends the chain, forming new DNA molecules.

The amount of DNA amplified through PCR increases significantly, allowing the virus to be detected. In the case of hepatitis B and C, PCR can detect very low quantities of the virus, making it superior to other diagnostic methods.

Hepatitis B and C: Epidemiology and Medical Importance: Hepatitis B and C are two major viral infections that can lead to liver disease, cirrhosis, and liver cancer. According to the World Health Organization (WHO), an estimated 296 million people globally are infected with hepatitis B, and 58 million people are infected with hepatitis C. These diseases often go unnoticed by patients, as symptoms may not appear until liver damage is severe. Therefore, early diagnosis of hepatitis B and C is critical to preventing serious complications. Achievements of PCR in Diagnosing Hepatitis B and C.

1. High Sensitivity and Specificity: PCR technology offers high sensitivity, allowing for the detection of even minimal amounts of the virus. This is crucial for detecting hepatitis B and C in the early stages. Additionally, because PCR is based on the viruses' DNA or RNA, it has a high level of specificity in detecting different strains.
2. Detection of Viral Load: PCR not only detects the presence of the virus but also quantifies its concentration (viral load). Viral load plays an important role in assessing the immune system's response and treatment efficacy. In the case of hepatitis C, a reduction in viral load indicates effective treatment with interferon and ribavirin.
3. Precise Confirmation of Hepatitis B and C: PCR technology enables precise confirmation of the genetic material of hepatitis B and C viruses. Hepatitis B virus DNA and hepatitis C virus RNA can be identified through PCR. This method is significantly more accurate than traditional serological methods, which detect only antibodies and may provide delayed responses.
4. Reducing Risks in Surgery and Organ Transplants: Early detection of hepatitis B and C in high-risk groups—such as organ transplant recipients or dialysis patients—allows for safe treatment before surgical procedures. Based on the viral load detected by PCR, antiviral treatments can be optimized for transplant patients.

Limitations and Drawbacks of PCR Technology

1. High Cost and Infrastructure Requirements: PCR technology is a high-cost and technologically complex method. It requires specialized laboratories, expensive reagents, and qualified personnel. In many developing countries, financial resources are insufficient to widely implement PCR tests, limiting diagnostic capabilities.
2. Mutations and Viral Variants: PCR tests rely on specific genetic sequences. Genetic mutations in viruses may result in new variants, potentially reducing the test’s effectiveness. For instance, hepatitis C has various genotypes, and some may not be accurately detected by PCR.
3. Sample Quality and False-Negative Results: The quality of sample collection and preservation is crucial for PCR tests. Poorly preserved or improperly handled samples can reduce the test's sensitivity and lead to false-negative results. This is particularly important for patients with low viral loads, where small amounts of viral DNA or RNA may go undetected.
4. Technical Complexity and Operational Challenges: PCR technology is complex and requires careful attention to technical details. The risk of contamination in laboratories is high, which can lead to inaccurate test results. The complexity of the PCR process means that it can only be operated by highly qualified specialists.

Comparison with Other Diagnostic Methods for Hepatitis B and C. Other diagnostic methods, such as ELISA (Enzyme-Linked Immunosorbent Assay) and rapid tests, are also available for detecting hepatitis B and C. These methods are often serological, detecting antibodies in the patient’s blood. However, PCR technology allows for molecular detection and direct confirmation of the virus. Serological methods may delay diagnosis, as antibodies are only produced after the immune system responds to the viral infection.

Recent Advances and Future Potential in PCR Technology: In recent years, several advancements have been made in PCR technology. For instance, real-time PCR (qPCR) has improved the ability to detect and monitor viral load more effectively. This method plays a crucial role in treating and monitoring patients' health. Additionally, efforts are being made to create faster and more sensitive tests for viral detection. The development of multiplex PCR, which can detect multiple viruses or pathogens simultaneously, is another promising advancement. This method could be highly beneficial in diagnosing co-infections, such as hepatitis B and C occurring together, or other liver-related diseases.

Conclusion: The use of PCR technology to confirm hepatitis B and C represents a significant step forward in the diagnosis and management of these infections. Its high sensitivity, specificity, and ability to quantify viral load make it a valuable tool for early diagnosis and monitoring of treatment efficacy. However, the high cost, infrastructure requirements, and challenges in dealing with viral mutations and sample quality pose limitations. Despite these drawbacks, continued advancements in PCR

technology hold the potential to further improve the accuracy and accessibility of hepatitis B and C diagnostics, ultimately leading to better patient outcomes. In the coming years, as the cost of PCR tests decreases and accessibility improves, we can expect broader implementation of this technology in both developed and developing countries, potentially leading to a significant reduction in hepatitis-related complications and fatalities.

Then the DNA polymerase enzyme gets to work and copies the single strand molecule, starting at the bound primer region. This final step, extension, is so-called because the DNA extends the DNA from the annealed primer and makes a complementary copy of the single strand. In practice, two different primers are used. A forward primer that binds to one strand and a reverse primer that binds to the opposite strand. The choice of primers is important as each primer binds the DNA in a specific place. The only DNA that is copied is the region between the forward primer binding location and the reverse primer binding location. The three PCR steps are repeated for around 30 or 40 cycles. Each cycle doubles the number of double stranded DNA molecules. Normally PCR is performed on a machine called a PCR thermal cycler or PCR machine. The PCR thermal cycler rapidly heats and cools the PCR reaction mixture thus allowing the denaturation, annealing and extension to occur. PCR Problems In practise, the PCR technique is more complicated than outlined above and it is not always simple to achieve the desired result. For example, primers can bind non-specifically to multiple places on the DNA strand. This results in a mixture of amplified product. In addition, various factors such as impurities in the DNA mixture, can prevent the reaction occurring at all. For further information see our section on PCR Troubleshooting Conventional gel-based PCR, or legacy PCR, as described above, has several disadvantages: it is labour-intensive, not easily automated or adapted for high throughput applications, and quantification of nucleic acids is arduous. The introduction of novel reagents, chemistries and instrumentation platforms has modified legacy PCR to create a new technology, real-time, fluorescence-based quantitative PCR (qPCR also known as real-time PCR or qRT-PCR) that addresses these issues. The use of real-time PCR allows monitoring of the progress of a PCR reaction in real time using fluorescent reporter molecules included in the PCR mixture. For further information see our section on Real-Time PCR

Take it out. PCR is a modern diagnostic method that allows you to identify any virus or bacterium in the body, even if their number is very small. PCR, which we have been using for many years, is used as a diagnostic method in cases where the number of viruses, such as hepatitis B, hepatitis C, is very important.

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