

## POLYMERASE CHAIN REACTION AND ITS VALUE IN THE DIAGNOSIS OF HERPES SIMPLEX COMPLEX

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The past few years have seen a profound revolution in biological sciences. The enormous advances in molecular biology are providing novel insights into the etiology and treatment of human diseases. In this article, the basic principles and techniques of the polymerase chain reaction and its significance in the diagnosis of herpes encephalitis are discussed to provide laboratory and medical staff with background information on the subject.

### Principles of the PCR

The polymerase chain reaction (PCR) was first reported in 1985,<sup>1</sup> and is an *in vitro* method of nucleic acid synthesis. Human  $\beta$  globin was one of the first DNA sequences to be amplified by PCR.<sup>1,2</sup> Since these early reports, numerous applications for PCR have been recorded. The introduction of heat-stable DNA polymerase<sup>2,3</sup> brought significant improvement to PCR, together with automated systems.

PCR is a technique which makes it possible to single out and multiply (amplify) a piece (sequence) of genetic information from among billions of other sequences to produce millions of copies. It has been applied to basic biological research, medical diagnostics, human genetics and forensic medicine. PCR amplifies minute pieces of genetic material, making it possible to study genetic material that is present in tiny amounts that cannot be directly detected or analyzed. With PCR, tiny quantities of bacteria or viruses can be identified in blood or other clinical samples on the basis of their genetic information.

PCR consists of a three-step process, known as a cycle, which is repeated many times, each repeat theoretically doubling the DNA target sequence. The three steps are denaturation, annealing and extension (Figure 1). In the first step of the reaction, denaturation, the two DNA strands are separated by heating the DNA to 94°C. This breaks the relatively weak hydrogen bonds between the nucleotide

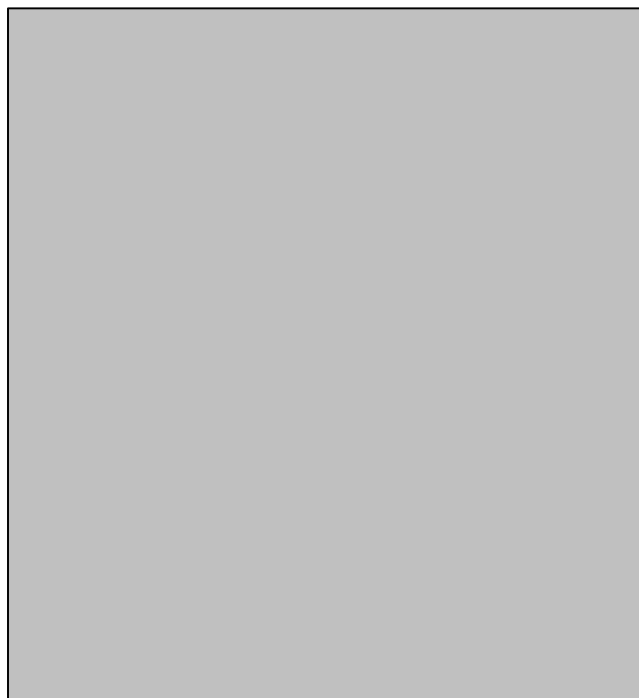


FIGURE 1. Schematic representation of the polymerase chain reaction (PCR).

strands. These rungs of the DNA ladder are, figuratively speaking, sawn in half, yielding two long, single strands. In the second step, known as annealing, two specific primers attach themselves to the complementary section of the DNA on the single strands. These primers are small, synthetically produced stretches of single-stranded DNA, usually about 20 to 30 bases long. They are selected so that one is complementary to the upper strand, and the other is complementary to the lower strand of the target sequence within the DNA. The annealing temperature is usually around 40°C to 60°C, and depends on the length and base sequence of the primers. Once the primers anneal, two short stretches of double-stranded DNA, required for the enzyme DNA polymerase, are generated. In the third step, or extension, these short stretches serve as starting blocks for the enzyme to extend the DNA, starting at the 3' end of the primer. The polymerase adds nucleotides complementary to the template DNA, thus extending the primers in the direction of the target sequence. This enzymatic reaction

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FIGURE 2. Polymerase chain reaction (PCR) amplification of DNA. A single target molecule is duplicated during every cycle, thus producing accumulation of an amplicon of a defined size.



FIGURE 3. Polymerase chain reaction (PCR) amplification of HSV-DNA (one primer set only). Lane 1, 1.5 kilobase DNA ladder, lane 2, negative control, lane 3, patient sample, lane 4, positive control.

produces two double strands from the two single strands of the original target DNA. This constitutes the end of the first cycle. The two new DNA double strands are identical (Figure 2). The number of target DNA copies approximately doubles at every cycle, producing an exponential increase in DNA, thus repetition of the cycle 25 times may produce more than 100,000 copies of a single target DNA molecule.<sup>2</sup>

For a viral RNA genome (e.g., HIV), amplification of RNA by PCR can be performed by annealing a primer to the RNA template, and then synthesizing a cDNA copy using reverse transcriptase (RT), followed by the PCR.

#### *Herpes Simplex Encephalitis (HSE)*

There is a strong association between herpes simplex encephalitis (HSE) disease and HSV-1. While meningitis is more often a complication of genital HSV-2 infection,<sup>4</sup> the largest proportion of cases of HSE are of HSV type 1. Between 1.6%<sup>5</sup> and 6.5%<sup>6,7</sup> of cases have been shown to be due to HSV-2. Herpes simplex encephalitis can occur at any age. The disease is known to affect both men and

women, with an estimated incidence of 1 in 250,000 to 1 in 500,000 persons per year.<sup>8</sup> A history of earlier HSV infection can be found in many patients, and HSE is, therefore, thought to be a disease which occurs due to the reactivation of latent virus. Some clinical manifestations of encephalitis include seizure, hallucinations, aphasia, personality alteration, memory defects and altered consciousness.<sup>9</sup> HSE is responsible for mortality of up to 70% if untreated, and up to two-thirds of survivors have residual neurological deficits which are sometimes severe.<sup>10</sup>

#### *Diagnosis of HSE*

The typical clinical picture of HSV primary infection or reactivation does not usually require virological confirmation. If necessary, the virus can be isolated directly from clinical materials such as vesicular fluid, throat swabs, washings, or corneal swabs. A differential between the two HSV types may be made by use of monoclonal antibodies specific for HSV-1 or HSV-2. Primary infection with HSV can be serologically identified by the detection of HSV-specific IgM antibodies.<sup>11</sup>

The major diagnostic problem is the early detection of HSE. The detection of HSV-specific intrathecal antibody production is not possible early in the course of disease, and recovery of viruses from cerebrospinal fluid (CSF) by conventional means has mostly been unsuccessful. Thus, the early diagnosis of HSE has been limited to the identification of HSV in brain biopsy tissue, a procedure that is usually avoided due to its invasive nature. Because of the problems associated with brain biopsy, more non-invasive methods which would enable a rapid diagnosis of HSE are being sought. A noninvasive approach, which overcomes the problems associated with brain biopsy, is to investigate the presence of markers of infection within the CSF.

Evidence of HSV DNA in brain biopsy material suggested that DNA technology could be applied to the analysis of CSF samples. Dot-blot hybridization of CSF gave positive results as early as day 2 after onset,<sup>12</sup> or when a more severe illness has developed.<sup>13</sup> However, the presence of HSV DNA in CSF was an important observation. With the introduction of PCR, which allows the amplification and detection of low numbers of DNA molecules, the potential for early detection of HSV DNA in the CSF became apparent.<sup>14-18</sup>

The value of the PCR in the acute phase diagnosis of HSE lies in its ability to detect nucleic acid at very low levels. The amount of viral nucleic acid present within CSF during the first 10 days after onset of neurological symptoms appears to be lower than the detection limits of other molecular techniques.

The argument over the utility of brain biopsy for diagnosis of HSE is now debatable. Application of herpes simplex virus polymerase chain reaction detection to CSF has been shown to be a sensitive and specific test for the

diagnosis of HSE.<sup>17,19</sup> In this test, two sets of oligonucleotide primers amplifying gene products from herpes simplex virus 1 and 2 are used (Figure 3). After amplification the DNA is separated by electrophoresis, and if a band characteristic of herpes simplex virus DNA is found, the test is considered positive. This test is positive quite early in the diagnosis and has a turnaround time that is less than 24 hours. The test generally remains positive during the first week of therapy.<sup>17</sup> This clearly obviates the need for consideration of a brain biopsy to confirm the diagnosis.

The PCR also has a value in the management of a patient with suspected HSE. In a study to evaluate the utility of an assay based on a PCR of CSF in the management of patients with suspected HSE, Tebas et al.<sup>20</sup> suggested that empiric therapy with acyclovir without laboratory confirmation was widely used. Therefore, in their model, they compared a PCR-based approach with empiric therapy that corresponds to current practice. Under very conservative assumptions, the PCR-based approach provided better outcomes, and in addition, resulted in substantially less acyclovir use. However, the use of DNA amplification techniques can be problematic. The techniques are susceptible to false-positive or false-negative results during the relatively complex sample processing,<sup>21</sup> therefore, careful handling of the test sample is essential.

#### Conclusions and Future Challenges

Central nervous system (CNS) infection caused by a virus whose complete genetic sequence was known and a virus which, until HIV, was the most researched of all viruses, could not, without invasive diagnostic procedures, be diagnosed with efficiency. Nucleic acid amplification procedures of increasing reliability and utility have been developed to detect viral DNA within the CSF. They are rapid, efficient, and minimally invasive. These procedures have also been conclusively validated. With this new efficiency in diagnosis, it is becoming increasingly evident that the presently accepted spectrum of CNS disease attributed to HSV must be expanded. With the diagnosis of an increased number of CNS infections comes a need for increased usage of antivirals, for example, in herpes meningitis. Antiviral therapy of apparently "benign" herpes meningitis is essential, since we do not know the potential long-term consequences of disease in the individual patient.

Application of nucleic acid amplification procedures is not without difficulty. These infections are comparatively rare, and a large number of non-HSV samples must be examined in order to detect the few positive samples. As a consequence, maintenance of quality standards is essential, and in the future, more widespread usage of external quality assurance measures will be mandatory.

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