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Major review

Principles and applications of molecular biology techniques for the microbiological diagnosis of acute post-operative endophthalmitis

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ABSTRACT

The systematic microbiological evaluation of endophthalmitis allows the confirmation of the infectious nature of the disease and the possible adaptation of treatment at the individual level and, at the collective level, the epidemiological characterization of the bacterial spectrum of endophthalmitis. Long reserved for research, the use of molecular biology techniques to complement conventional culture techniques has become important for the diagnosis of endophthalmitis in recent years. These new diagnostic techniques are particularly useful for the microbiological study of bacteria that are difficult or impossible to grow because of their intrinsic properties, their presence in only a small inoculum, their sequestration on prosthetic materials, or their inactivation by prior antibiotic treatment. These techniques are based on the polymerase chain reaction (PCR), which allows the amplification and detection of extracted bacterial deoxyribonucleic acid (DNA) that is initially present in minute quantities in an ocular sample. In practice, these conventional or real-time PCRs allow either the a priori detection of bacterial DNA (universal PCR) or the identification of a specific DNA fragment of a bacterial genus or species (specific PCR). New techniques of PCR will allow more rapid bacterial identification and also characterization of genotypic properties, such as genes of virulence or antibiotic resistance.

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1. Introduction

The therapeutic management of patients with postoperative endophthalmitis has changed little since the publication of the Endophthalmitis Vitrectomy Study results in the mid 1990s.[35] Instead, the last decade has been marked by the development of new techniques of microbiological detection and identification that are complementary to conventional culture techniques, resulting in considerable progress in the diagnosis of these intraocular infections. The identification of the causative germ in endophthalmitis has several advantages, including quick confirmation of the infectious nature of the inflammation and the possibility to adapt the medical and surgical therapeutic management to the bacterial virulence. In preventive care, the modern microbiological approach should also allow the reassessment of surgical hygiene procedures and better define the epidemiology of the disease. The infectious agent responsible for postoperative endophthalmitis is usually a bacterium from the patient's own conjunctival flora that enters the eye through the incision during or after the surgery. More rarely, particularly at certain latitudes, a fungus may be involved. Because the infection rarely diffuses through the sclera, the conventional microbiological diagnostic approach is based on the culture of ocular samples of the aqueous or vitreous humor. The bacterial agent is identified in approximately 22–30% of cases upon culture of aqueous humor and in 40–69% of the cases upon analysis of vitreous samples.[3,18,26,30,35] The limited sensitivity of culture techniques is explained by several factors, such as the low causal bacterial inoculum, the sequestration of microorganisms on solid surfaces (intraocular lens, crystalline capsule, crystalline residues), the prior use of antibiotics, and/or the involvement of bacteria that are slow or difficult to culture.[32] The use of pan-bacterial or specific polymerase chain reaction (PCR) techniques as a complement to conventional culture techniques improves the microbiological diagnostic yield in endophthalmitis and might change the diagnosis and treatment strategy for this disease.

2. Fundamental principles of the PCR technique

PCR, a molecular biology technique developed relatively recently that is useful for the microbiological diagnosis of infections, is based on the amplification and subsequent detection of bacterial deoxyribonucleic acid (DNA) in a biological sample. DNA is a polymer of nucleotides (polynucleotide), each of which consists of a phosphate group associated with a sugar (deoxyribose) that is linked to a nitrogenous base in a double helix structure. Four bases are found in DNA: adenine, thymine, cytosine, and guanine.[41] A strand of DNA is formed by the ordered repetition of these nucleotides. The nitrogenous bases form complementary pairs: adenine associates with thymine and guanine with cytosine. The second DNA strand is therefore complementary to the first, and the paired nitrogenous bases are linked through hydrogen bonds to form base pairs. The order of succession of these four nucleotides throughout the genome is specific to each individual and forms the genetic code that is the basis of protein synthesis. At each cell division, the double-stranded DNA molecule is replicated into two double-stranded daughter DNA molecules. During replication, the double helix is first denatured by breaking the hydrogen bonds between the DNA strands. A replication fork then forms, resulting in two distinct single-stranded DNA molecules. Each of these strands is copied by the action of DNA polymerases, which associate the complementary nucleotide to each base to form two new double-stranded DNA molecules identical to the original molecule (Fig. 1).

PCR exploits these properties in vitro to copy a specific DNA fragment using two main principles:

- The hybridization and dehybridization (denaturation) properties of the two complementary DNA strands, depending on the temperature.
- The ability of DNA polymerases to synthesize a complementary DNA strand based on a primer hybridized specifically to a DNA template.

Developed by Kary Mullis in 1985, leading to the Nobel Prize in Chemistry in 1993,[29] PCR is an in vitro technique of targeted replication consisting of a sequence of repetitive thermal cycles, during which the number of the target DNA molecules used as templates is doubled in each cycle. The products obtained at the end of each cycle are used as templates for the next cycle, resulting in exponential amplification. Therefore, from a small biological sample, it is possible to amplify a given DNA fragment that is initially present in only small amounts and obtain a quantity sufficient for detection.

2.1. *Classic or conventional PCR amplification*

2.1.1. *Reaction mixture composition*

The buffered reaction medium includes (Fig. 1):

a. The template DNA previously extracted from the sample for analysis. This DNA is denatured (separated into two complementary strands) by heating the sample to 94°C.
b. The two specific primers for the target DNA sequence are sense and antisense. These primers are synthetic oligonucleotides measuring 15 to 30 bases in length that are complementary to the target DNA strand and its complementary strand, respectively. These primers are selected to frame the DNA sequence to be amplified. In the field of microbiological diagnosis, these primers can target a genetic sequence common to all bacteria (universal or pan-bacterial PCR) or a sequence that is specific to a bacterial genus or species (specific PCR).
c. The Taq polymerase, which is a thermostable DNA polymerase extracted from the bacterium *Thermus aquaticus*. The temperature optimum of this polymerase is 72°C, but it can maintain activity through repeated cycles at 95°C, which has enabled the automation and expansion of PCR.
d. The trinucleotide precursors: dGTP (guanine), dATP (adenine), dCTP (cytosine), and dTTP (thymine) or dUTP (uracil), collectively referred to as dNTPs (deoxynucleotide triphosphates).