# Chapter 4
# The Molecular Diagnosis of Endophthalmitis

**Christophe Chiquet, Sandrine Boisset, Pierre-Loïc Cornut, and Max Maurin**

## 4.1 Introduction

The incidence of acute postoperative endophthalmitis is low and varies depending on the type of eye surgery: approximately one case for every 1000–2000 cataract surgeries [1]. The causal infectious agent is a bacterium in most cases. Endophthalmitis requires rapid microbiological investigations to confirm the diagnosis and aggressive treatment, including intravitreal administration of antibiotics and in 30–60 % of patients a therapeutic vitrectomy. Identification of the microorganism involved is important for several reasons: to quickly confirm the infectious nature of inflammation, to justify and adapt the intravitreal antibiotic therapy, to rationalize the surgical decision for therapeutic vitrectomy, to precisely determine the epidemiology of the disease, and to reevaluate surgical hygienic procedures. While the clinical criteria for diagnosis of endophthalmitis have not evolved in recent years (decreased visual acuity in an inflamed and often painful eye), the microbiological diagnosis has benefited from advances in molecular biology techniques allowing rapid detection and identification of human pathogens.

C. Chiquet, MD, PhD (✉)
Department of Ophthalmology, Grenoble Alpes University, Grenoble, France

Department of Ophthalmology, University Hospital of Grenoble, Grenoble, France
e-mail: christophe.chiquet@inserm.fr

S. Boisset, PharmD, PhD • M. Maurin, MD, PhD
Department of Microbiology, Grenoble Alpes University, Grenoble, France

Department of Microbiology, University Hospital of Grenoble, Grenoble, France

P.-L. Cornut, MD, PhD
Department of Ophthalmology, University of Lyon, Lyon, France

Department of Ophthalmology, University Hospital, Lyon, France



M.L. Durand et al. (eds.), *Endophthalmitis*, DOI 10.1007/978-3-319-29231-1_4

### *4.1.1 Intraocular Samples*

#### 4.1.1.1 Sample Collection

Intraocular samples, i.e., aqueous and vitreous, must be obtained after local antisepsis. The French Institutional Endophthalmitis Study (FRIENDS) group recently reported that pan-bacterial polymerase chain reaction (PCR) testing (i.e., 16S rRNA gene amplification and sequencing) has comparable sensitivity when testing diluted or undiluted vitreous [2]. Collecting diluted vitreous is easier to perform and does not induce hypotony, therefore limiting the risk of choroidal hemorrhage, retinal detachment, or displacement of the infusion cannula (as compared with undiluted vitreous sampling).

#### 4.1.1.2 Sample Processing and Storage

The sample collected for PCR testing should be placed in a sterile screw-capped DNA-free tube. The minimum volume for molecular analysis is approximately 50 µl. This tube should be placed in a secondary sterile container. The delivery time of the sample to the microbiology laboratory must be as short as possible and should not exceed 2 h at room temperature. If these conditions cannot be fulfilled, the PCR tubes should be stored at 4 °C for 48 h or −20 °C for longer periods [3].

### *4.1.2 Molecular Techniques for the Diagnosis of Endophthalmitis*

In recent years, a number of PCR-based assays have been implemented in microbiology laboratories for routine diagnosis of infectious diseases. Although the culture methods remain the gold standard because of their high specificity and the possibility to test the susceptibility of isolated pathogens to antibiotics, their sensitivity may be low, especially for fastidious and slow-growing microorganisms. In endophthalmitis patients, molecular methods provide a more rapid and sensitive diagnosis [4–6]. PCR-based techniques may also be used to detect viral or fungal nucleic acids (DNA or RNA) [7, 8].

PCR amplification of DNA usually requires three steps: total DNA extraction from clinical samples, target DNA amplification using specific primers, and a post-PCR step to identify the amplified products (Fig. 4.1). Steps 2 and 3 are combined for real-time PCR, reducing the turnaround time of the procedure (60–90 min versus 120–180 min). Whatever DNA amplification method is used, a number of controls are needed to ensure the accuracy of the results, including a DNA extraction control (proper DNA extraction), a negative amplification control (no false-positive results), a positive amplification control (no false-negative results), and an internal control (no DNA polymerase inhibitors). It should be mentioned that many PCR tests use

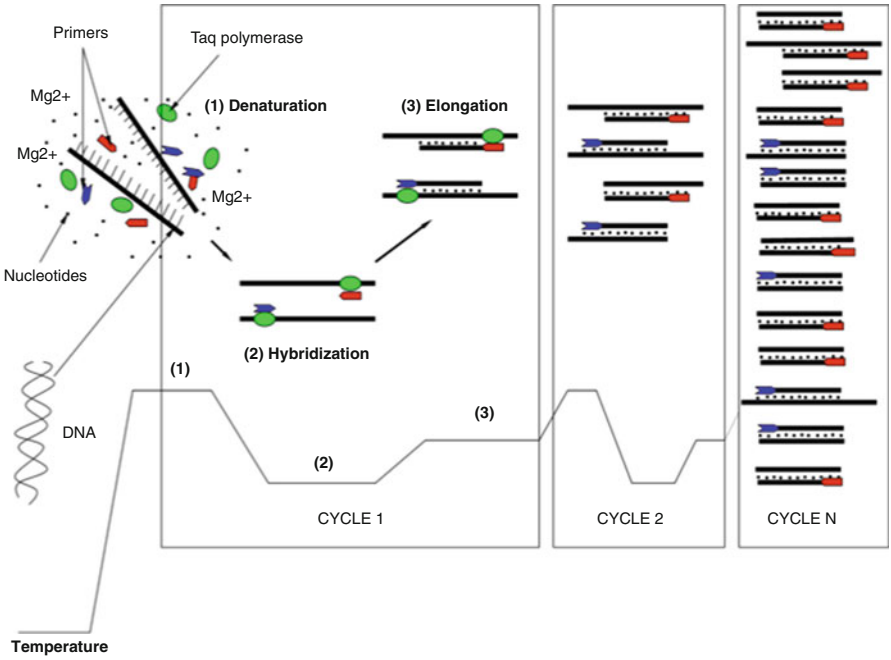


**Fig. 4.1** Principle of the polymerase chain reaction (PCR) assay

amplification of the human β-globin gene present in all eukaryotic cells as a specific internal control, but this may not be appropriate for intraocular samples, which often contain few eukaryotic cells. It should also be emphasized that molecular diagnostic tools currently available in clinical laboratories for etiological diagnosis of endophthalmitis are often made in-house and thus require careful validation prior to their clinical use. Development of a few commercial tests would be useful for the molecular diagnosis of endophthalmitis.

#### 4.1.2.1 Pan-bacterial Conventional PCR

This technique is based on amplification of the 16S rRNA gene (encoding the small subunit of bacterial ribosomal DNA) using universal primers complementary to DNA regions that are conserved among almost all bacterial species (Fig. 4.2). The use of pan-bacterial PCR for aqueous and vitreous humors has been described by several authors [4, 9–11]. Conventional PCR is typically used for amplification of the 16S rDNA. A precise identification of the bacterial species involved requires a post-PCR step, which often corresponds to the sequencing of the amplified DNA (Fig. 4.3) and its comparison to DNA sequences contained in large databases (e.g., GenBank). This step uses DNA sequence alignment programs such as the BLASTN program of the National Center of Biotechnology Information (NCBI, USA,

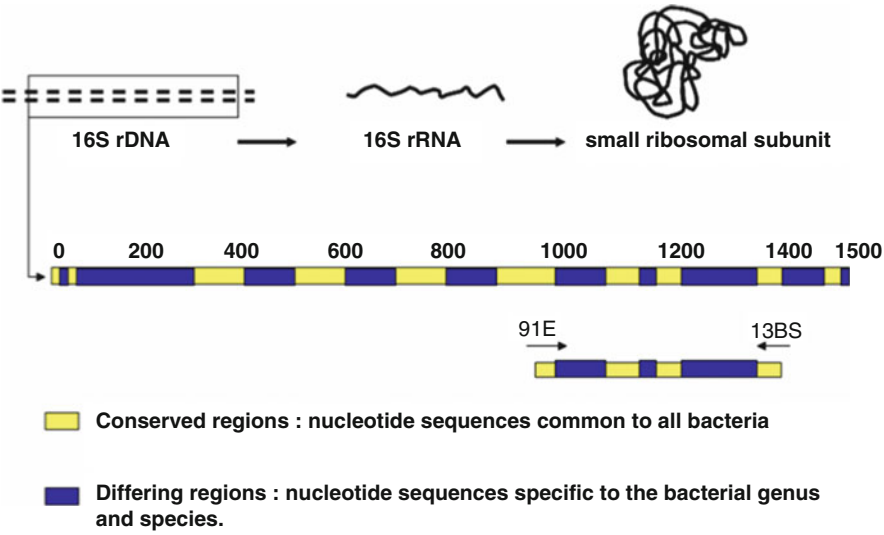


**Fig. 4.2** Scheme of the 16S rRNA gene (1500 nucleotides). Conserved nucleotide sequences in Eubacteria alternate with variable nucleotide sequences specific for bacterial genera or species

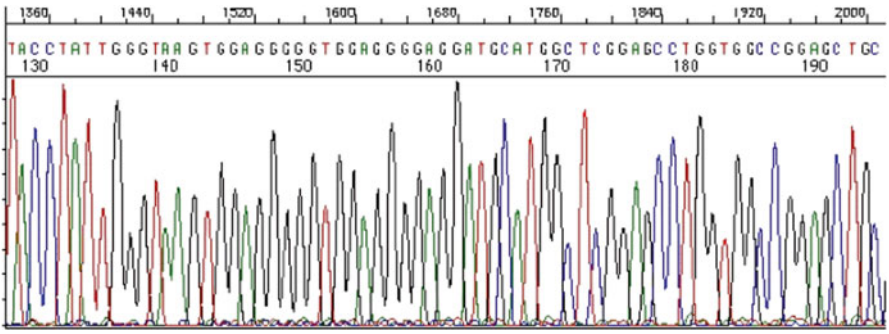


**Fig. 4.3** DNA sequencing: chromatogram obtained using an automated DNA sequencing procedure. Each DNA fragment is complementary to the target DNA and contains a nucleotide labeled with a specific fluorophore for each nucleotide type (A, T, C, or >G). These fragments are separated using acrylamide gel electrophoresis, with subsequent detection of the terminal labeled nucleotide

http://blast.ncbi.nlm.nih.gov/) or phylogenetic programs (e.g., Quick BioInformatic Phylogeny of Prokaryotes, Lyon University, France, http://umr5558-sud-str1.univ-lyon1.fr/lebibi/lebibi.cgi) [4, 11–15]. The QBPP (formerly BIBI) software was designed to automate DNA sequence analysis for bacterial identification in the clinical field. Species identification is considered to be reliable when the percentage of similarity between the analyzed 16S rDNA sequence and the sequences deposited in databanks is at least 98 % [16]. A phylogenetic approach (Fig. 4.4) is now often used [16, 17].

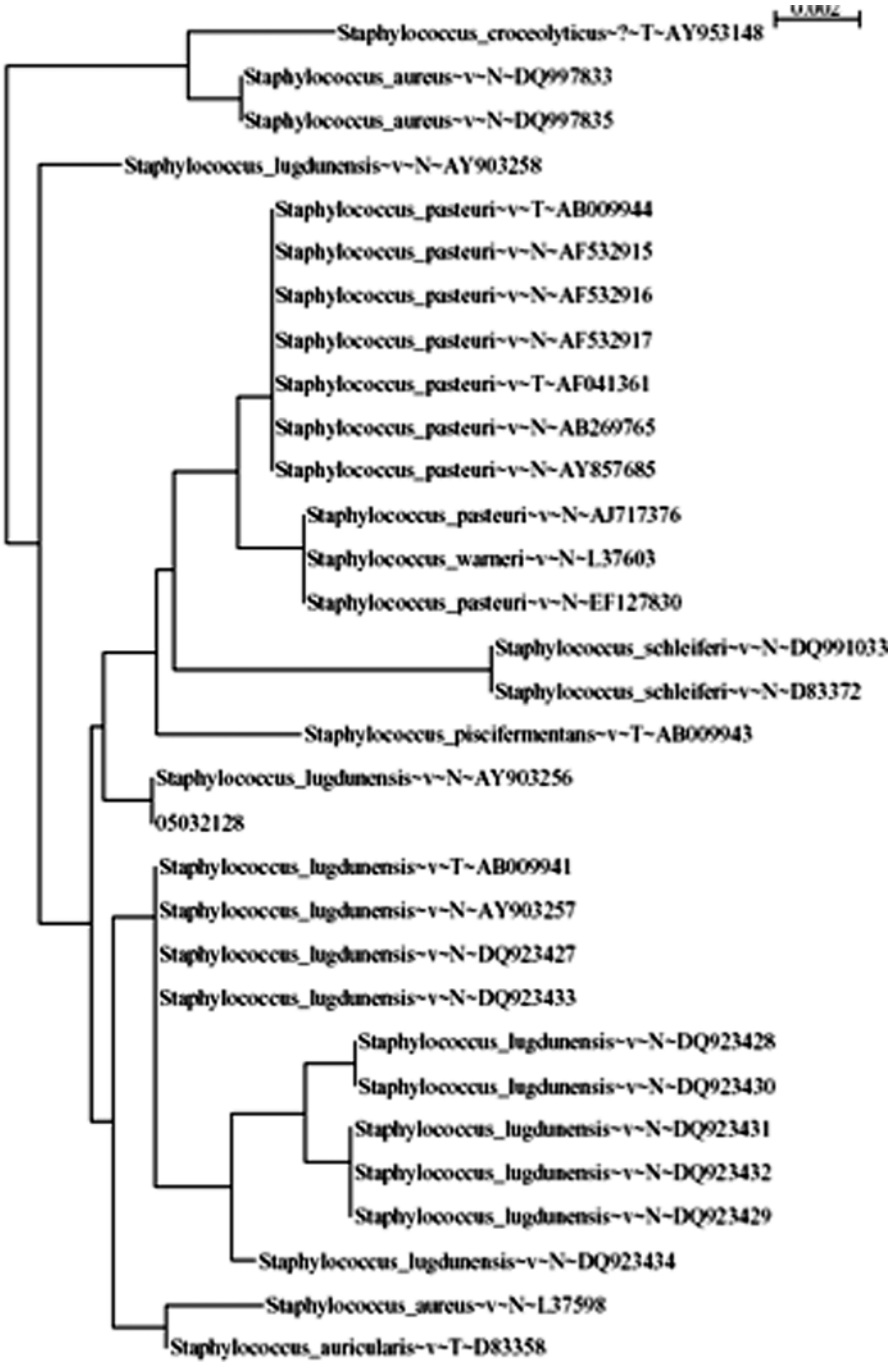


**Fig. 4.4** A phylogenetic tree obtained after DNA sequence alignment and analysis and determination of a sequence similarity index

The use of alternative methods to DNA sequencing is now much less common. The amplified DNA may be hybridized with a fluorophore-labeled probe, which can specifically identify a bacterial group (e.g., gram-positive or gram-negative bacteria), a bacterial genus, or a bacterial species [18–20]. Pan-bacterial PCR may be followed by a "nested PCR" (see below) using specific primers to quickly distinguish gram-positive from gram-negative bacteria [10, 18, 19]. Restriction fragment length polymorphism (RFLP) methodology is based on the use of restriction enzymes that cut DNA at specific sequences (restriction sites). The resulting DNA fragments are then separated by gel electrophoresis, and species identification is based on specific restriction patterns [15].

Pan-bacterial PCR has the advantage of covering the entire bacterial spectrum. It is particularly useful when a large panel of bacterial species may cause the same disease, such as in endophthalmitis patients. The limitations of this technique include lower sensitivity and specificity as compared to species-specific PCR and more importantly a long turnaround time (2–3 days is required for species identification). The sensitivity can be slightly improved by performing a second round of PCR amplification using amplified products obtained after the primary PCR reaction. This technique, referred to as nested PCR, significantly increases the risk of false-positive results due to exogenous DNA contamination. Different species may share similar 16S rDNA sequences (e.g., *Streptococcus mitis* and *Streptococcus pneumoniae*), and their differentiation requires further identification tests [21]. False-positive results may occur due to contamination of clinical samples with exogenous DNA. The pan-bacterial PCR assay is mainly useful when infection is caused by a single bacterial species, which is often the case in endophthalmitis patients.

For samples with a polymicrobial flora, the mixture of 16S rDNA sequences obtained is more difficult to analyze. In this case, amplified DNA from PCR reactions must be cloned into a plasmid to aid sequencing and to establish the identity of individual PCR products in samples with mixed populations of 16S rDNA [15]. This technique is tedious and rarely performed on a routine basis.

Finally, antibiotic susceptibility testing of the bacteria involved requires their isolation in culture. Only a few resistance gene determinants can be detected using PCR.

It should be noted that a similar procedure may be implemented for fungal pathogens, by amplification and sequencing of the 18S rRNA or 28S rRNA coding genes (fungal ribosomal RNA molecules). This diagnostic approach, which can be referred to as the pan-fungal PCR, is less commonly used than for the detection of bacteria [8, 22]. Fungal PCR assays are more prone to giving false-positive results than bacterial assays because of an increased risk of exogenous contamination and therefore may be more difficult to interpret.

#### 4.1.2.2 Pan-bacterial Real-Time PCR

Recent studies have reported the use of real-time PCR rather than conventional PCR for rapid detection of bacterial 16S rDNA [11, 20, 23]. Real-time PCR combines a PCR amplification of target DNA with simultaneous detection of the amplified PCR products using fluorescent reporter molecules, which may be dyes that bind to the

double-stranded DNA (e.g., SYBR® Green) or sequence-specific probes (e.g., TaqMan® Probes). The PCR amplification process can be monitored in real time by measuring the progressive increase in the fluorescence emitted by the reporter molecules. This process has a shorter turnaround time than conventional PCR because it eliminates the postamplification step.

The real-time PCR technology may also be used for rapid detection and differentiation of large groups of microorganisms. Bispo et al. [24] described two coupled real-time PCR reactions for the detection and differentiation of gram-positive and gram-negative bacteria causing endophthalmitis.

#### 4.1.2.3 Specific PCR and Real-Time PCR

Specific and real-time PCR tests have been developed for the detection of specific pathogens. While the PCR technique uses pathogen-specific primers (complementary to a specific region of the target pathogen), most real-time PCR tests also include specific probes, increasing the specificity of detection and identification of the target microorganisms. Specific real-time PCR tests are also easier to implement in clinical microbiology laboratories and are usually more rapid and sensitive than PCR assays [20]. Both techniques may allow the detection of a specific bacterial genus or species (e.g., all *Staphylococcus* species or *Staphylococcus aureus*, respectively).

The main drawback of specific PCR or real-time PCR methods is the need for oriented diagnosis (a priori search for a bacterium). Therefore, these tests are usually combined with pan-bacterial PCR testing. In endophthalmitis patients, real-time PCR assays are mainly used for early detection of the most virulent species (e.g., *S. aureus* and *S. pneumoniae*) and fastidious or slow-growing species. As an example, Therese et al. developed a specific PCR targeting *Propionibacterium acnes* [25].

#### 4.1.2.4 Multiplex PCR and Real-Time PCR

To enhance the cost effectiveness ratio of the molecular tests, a rational approach would be the use of multiplex PCR or real-time PCR, which are variants of these techniques allowing simultaneous detection of multiple DNA targets in a single reaction. Goldschmidt et al. [20] reported the use of a multiplex real-time PCR assay allowing simultaneous detection of several genera (*Staphylococcus*, *Streptococcus*, *Haemophilus*, *Pseudomonas*, *Acinetobacter*, *Corynebacterium*) and families (*Enterobacteriaceae* and *Propionibacteriaceae*).

#### 4.1.2.5 Quantitative Real-Time PCR

When using real-time PCR technology, a threshold cycle can be determined as the number of amplification cycles required for the fluorescent signal to cross a predefined threshold. Using a calibration curve, the threshold cycle can give an estimation of the amount of target DNA present in the clinical sample before amplification.

Sugita et al. [11, 26] used quantitative real-time PCR to diagnose bacterial endophthalmitis. They detected a high number of bacterial genome units in ocular samples (from $1.7 \cdot 10^3$ to $1.7 \cdot 10^9$ genome units/ml). Determination of the bacterial load in intraocular samples could help differentiate true infection from exogenous contamination when samples are collected. Melo et al. [27] defined a cutoff threshold cycle differentiating infection from contamination, by testing intraocular samples from patients with proven bacterial endophthalmitis and aqueous samples obtained at the end of cataract surgeries taken as controls. Using a broad-range PCR, a threshold cycle value between 19.5 and 34.5 was compatible with bacterial endophthalmitis, while a threshold cycle value of 39 was found for the two contaminated aqueous humor samples.

#### 4.1.2.6 Reverse Transcriptase PCR (RT-PCR)

In the reverse transcriptase assay, DNA amplification by PCR is preceded by a reverse transcription reaction in order to produce complementary DNA from RNA. Bacterial mRNAs have been proposed as markers for cell viability since they are very unstable molecules with very short half-lives inside the cell. Thus, the detection of mRNAs indicates that the bacterium is alive and metabolically active. Aarthi et al. [28] developed an RT-PCR assay targeting the 16S rRNA to determine the bacterial viability in intraocular specimens.

#### 4.1.2.7 DNA Microarray

DNA microarray analysis is a molecular method that simultaneously detects and identifies a wide variety of genes in a single experiment. In the study conducted by Sakai et al., 76 pathogen-specific probes were fixed on a chip to hybridize labeled PCR products amplified from clinical samples. This microarray assay, previously developed to detect and identify 76 bloodstream infection-associated pathogens (bacteria and fungi) in blood samples, was applied to ocular samples collected from patients with clinically diagnosed endophthalmitis [29]. The main drawback of this technology is that a limited number of microarray assays for diagnostic purposes are commercially available, and these tests are usually very costly.

### *4.1.3 Contribution of PCR-Based Assays to the Diagnosis of Postoperative Endophthalmitis*

In patients with acute postoperative bacterial endophthalmitis, gram-positive bacteria are predominant: 85 % of the microorganisms involved in the French GEEP study (group of epidemiologic and prophylactic studies) [30], 94.1 % in the American Endophthalmitis Vitrectomy Study [31–33], and 97 % in the French

multicenter study of the FRIENDS group [4]. Among these gram-positive bacteria, *Staphylococcus epidermidis* predominated (45–50 %), followed by streptococci (24–37.7 %) and *S. aureus* (7.5–11.5 %) [4]. Gram-negative bacteria (e.g., *Escherichia coli*, *Proteus*, *Klebsiella*, *Serratia*, and *Pseudomonas* species) account for 3–15 % of culture-positive endophthalmitis cases [4, 30]. Polymicrobial infections have been described in several studies, with a frequency varying from 0 to 29 % [34, 35]. In our experience, coinfection is rare in this type of endophthalmitis.

The use of PCR for microbiological diagnosis of endophthalmitis was first reported in 1994 [36]. The studies published since 1994 are summarized in Table 4.1. They show that molecular biology techniques are useful for diagnosis of acute [4, 10, 11, 13, 18–20, 23, 25, 38] and chronic endophthalmitis [14, 19, 23, 25, 36, 38].

Most of the studies published have used conventional pan-bacterial PCR [13, 15, 19, 25, 36–38] (Table 4.1). Identification of the genus and species from conventional pan-bacterial PCR has not been systematically reported [25, 36–38]. When identification was attempted, in most studies this post-PCR stage was performed by sequencing [10, 13–15], more rarely using restriction fragment length polymorphism (RFLP) [15], gram-positive/gram-negative nested PCR [19], or hybridization of specific gram-positive/gram-negative waves [18]. In a recent review [41], we reported that an analysis from 16 studies shows a 40.5 % identification rate for conventional culture (193 positive samples out of 476) and 82.3 % for PCR (451/548 positive samples); the number of false-positives remains very low, limited to 3 % (9/296 control samples).

The FRIENDS group reported the sensitivity of pan-bacterial PCR in 100 acute endophthalmitis cases following cataract surgery; for aqueous samples, this sensitivity was similar to that of conventional culture (35 % versus 38 %) [4]. However, the combination of the two techniques allowed identification of a bacterial species in 47 % of cases. The identification rate increased to 68 % for vitreous samples when combining PCR and culture, while comparable sensitivity values were found for culture (54 %) and PCR (57 %) alone. When all of clinical samples were considered, pan-bacterial PCR was positive in 87 % of patients, and 25 % of patients had a positive PCR test but negative cultures. In addition, if the results of cultures were not taken into account, PCR only would have made the diagnosis in 61 % of the cases. Thus, pan-bacterial PCR and traditional cultures are actually complementary diagnostic methods for the etiological diagnosis of postoperative endophthalmitis. Pan-bacterial PCR had much higher sensitivity than culture when vitreous samples were collected following one or more intravitreal injections of antibiotics (70 % versus 9 % sensitivity, respectively) [4]. Additionally, pan-bacterial PCR has the same sensitivity for diluted vitreous than for undiluted vitreous samples collected during pars plana vitrectomy [2]. Results of the FRIENDS group study also indicated that, for a given patient, there was no need to repeat bacteriological analyses if PCR and culture testing of the first collected intraocular samples were both negative.

Broad-range real-time PCR for bacteria measures the amplification of the target rDNA genes [11]. This technique provided a diagnosis in 64 % of the cases. Since this PCR allows quantification of bacterial load, it can be used to distinguish contamination and infection on cycle threshold values [27].

**Table 4.1** Results of major studies using PCR in endophthalmitis

| Study | Population studied, samples/ infection category | Technique | Results |
|---|---|---|---|
| Hykin et al. [36] | 19 patients<br>19 chronic postoperative endophthalmitis<br>23 samples (V from PPV)<br>29 negative controls (V) | Phenol chloroform DNA extraction<br>*Nested PCR* with 16S rDNA primers, *no sequencing mentioned* | Culture +: 39 % (9/23)<br>16S rDNA PCR +: 74 % (17/23)<br>16S rDNA PCR + for controls: 14 % (4/29)<br>Sensitivity of the 16S rDNA primers: 50 fg (approximately 10 genome copies) of *S. epidermidis*<br>*P. acnes* PCR + in vitreous: 35 %<br>*P. acnes* PCR + in controls: 0 % |
| Lohman et al. [10] | 16 patients<br>10 acute and 6 chronic postoperative endophthalmitis<br>32 samples (16 A, 16 V from PPV)<br>20 controls (10 A, 10 V) | DNA extraction with QIAamp tissue kit (Qiagen)<br>*PCR sequencing, hybridization with probes specific for gram + and –* | Culture +: 25 % (8/32)<br>In A: 6.2 % (1/16); in V: 43.7 % (7/16)<br>16S rDNA PCR +: 93.7 % (30/32)<br>In A: 100 % (16/16)<br>In V: 87 % (14/16, negative in 2 chronic cases)<br>Correlation with culture: 100 % for positive results<br>16S rDNA PCR + in controls: 0 % |
| Therese et al. [25] | 55 patients<br>Acute and chronic, exogenous and endogenous, bacterial and fungal endophthalmitis<br>58 samples: 28 A, 30 V from PPV)<br>20 controls (6 A, 14 V) | Phenol chloroform DNA extraction<br>*Nested PCR, no sequencing mentioned 16S rDNA primers*<br>*Nested PCR with P. acnes-specific primers* | Culture +: 46.5 % (27/58) (20 bacteria and 7 fungi)<br>16S rDNA PCR +: 63.8 % (37/58)<br>16S rDNA PCR + in controls: 5 %<br>Sensitivity of the 16S rDNA PCR: 1 pg after round 1, 40 fg after round 2<br>*P. acnes* PCR +: 52.9 % (9/17 16S rDNA PCR + with culture – samples)<br>*P. acnes* PCR + in controls: 0 %<br>Sensitivity of the *P acnes* primers: 1 pg after round 1, 50 fg after round 2 |

| | | | |
|---|---|---|---|
| Okhravi et al. [15] | 25 patients<br>37 samples (15 A, 22 V)<br>38 controls (19 A, 19 V) | Phenol chloroform DNA extraction<br>*Nested PCR, bacterial identification by RFLP and DNA sequencing*<br>*Cloning and sequencing for polymicrobial infection* | Culture +: 54 % (20/37)<br>In A: 33 % (5/15); in V: 68 % (15/22)<br>16S rDNA PCR +: 100 % (37/37)<br>Correlation with culture: 100 % for positive results<br>6 unidentified rDNA sequences due to poor sequence quality or polymicrobial infection<br>16S rDNA PCR + in controls: 5 % (2/38, 1 A, 1 V) |
| Anand et al. [18] | 55 patients<br>29 postoperative (16 acute, 13 delayed), 22 post-traumatic, 4 endogenous endophthalmitis<br>57 samples (17 A, 40 V)<br>25 controls (10 A, 15 V) | Phenol chloroform DNA extraction<br>*Conventional PCR, hybridization with gram + and gram − probes* | Culture+: 56.1 % (32/57)<br>In A: 47 % (8/17); in V: 60 % (24/40)<br>16S rDNA PCR +: 91.2 % (52/57)<br>In A: 88 % (15/17; 5 g+ and 10 g−)<br>In V: 92 % (37/40; 19 g+ and 20 g−)<br>Correlation with culture: 100 % for positive results<br>2 vitreous samples positive for both gram+ and gram−<br>Sensitivity of the PCR-DNA probe hybridization evaluated on a range of common pathogens: 30 fg of DNA<br>16S rDNA PCR + in controls: 0 % |
| Lohmann et al. [14] | 25 patients<br>Chronic post-cataract endophthalmitis<br>50 samples (25 A, 25 V)<br>20 controls (10 A, 10 V) | DNA extraction: QIAamp tissue kit (Qiagen)<br>*PCR sequencing*<br>*16S rDNA primers and pan-fungal primers* | Culture +: 12 % (6/50)<br>In A: 0 % (0/25); in V: 24 % (6/25)<br>16S rDNA PCR +: 88 % (44/50)<br>In A: 84 % (21/25); in V: 92 % (23/25)<br>16S rDNA PCR + in controls: 0 % |
| Bagyalakshmi et al. [37] | 30 patients<br>Post-cataract (acute 9, chronic 3), post-trauma (1), endogenous (1) endophthalmitis<br>30 samples (19 A, 11 V)<br>Comparative results of the different methods available in 14 samples (8 A and 6 V) | DNA extraction: Qiagen kit<br>*Nested–multiplex PCR*<br>*16S rDNA primers, P. acnes primers, pan-fungal primers*<br>For pan-bacterial and *P. acnes* PCR, a second PCR was performed (nested PCR) | Culture +: 21.5 % (3/14)<br>In A: 25 % (2/8); in V: 16.7 % (1/6)<br>16S rDNA PCR +: 85.7 % (12/14)<br>In A: 87.5 % (7/8); in V: 83.3 % (5/6)<br>Pan-fungal PCR +: 14.3 % (2/14, 2 samples 16S rDNA PCR −)<br>*P. acnes* PCR +: 28.6 % (4/14)<br>Sensitivity of the multiplex PCR for detection of eubacterial and *P. acnes* genome: 100 fg |

(continued)

**Table 4.1** (continued)

| Study | Population studied, samples/ infection category | Technique | Results |
|---|---|---|---|
| Chiquet et al. [12] | 30 patients<br>Acute and delayed-onset endophthalmitis<br>44 samples (28 A, 16 V from PPV)<br>40 controls (30 A, 10 V) | DNA extraction with Qiagen kit<br>*PCR sequencing*<br>*16S rDNA primers*<br>*91E*: *TCAAAKGAATTGACGGGGGC*/*13BS*: *GCCCGGGAACGTATTAC* | Culture +: 31.8 % (14/44)<br>In A: 39.3 % (11/28); in V: 18.7 % (3/16)<br>16S rDNA PCR +: 61.4 % (27/44)<br>In A: 60.7 % (17/28); in V: 62.5 % (10/16)<br>Correlation with culture: 100 % for positive results<br>Sensitivity of the PCR for detection of *S. epidermidis* genome: 500–1000 pathogens<br>Culture or 16S rDNA PCR + in controls: 0 % |
| Chiquet et al. [4] | 100 patients<br>Acute post-cataract endophthalmitis<br>246 samples<br>114 initial samples (IS) collected before intravitreal antibiotics (76 A, 38 V from biopsy)<br>132 secondary samples (SS) after intravitreal antibiotics (62 A, 70 V from biopsy or PPV)<br>60 controls (35 A, 25 V) | DNA extraction with Qiagen kit (Qiagen)<br>*PCR sequencing*<br>*16S rDNA primers* | Culture +: 43.7 % (45/103) in IS; 12.6 % (15/119) in SS<br>In A: 38.2 % (26/68) in IS; 19.6 % (10/51) in SS<br>In V: 54 % (19/35) in IS; 7.3 % (5/68) in SS<br>16S rDNA PCR +: 41.9 % (47/112) in IS;<br>54 % (67/124) in SS<br>In A: 34.6 (26/75) in IS; 29 % (16/55) in SS<br>In V: 56.7 % (21/37) in IS; 73.9 % (51/69) in SS<br>Correlation with culture: 100 % for positive results<br>Culture or 16S rDNA PCR + in controls: 0 % |
| Sowmya and Madhavan [38] | 72 patients<br>45 post-cataract (21 acute, 14 delayed, 10 chronic), 16 post-trauma, 11 endogenous endophthalmitis<br>144 samples (72 A, 72 V), intravitreal antibiotics before sampling in most cases | DNA extraction: AccuPrep® Genomic DNA extraction kit (Bioneer)<br>*Nested PCR*, *no sequencing mentioned*<br>16S rDNA primers | Culture +: 37.5 % (27/72 patients) 24 bacteria and 3 fungi<br>16S rDNA PCR +: 84 % (121/144 samples) (corresponding to 100 % of patients, 24 polymicrobial cases with mixed sequences)<br>In A: 77.7 % (56/72); in V: 90.2 % (65/77) |

| | | | |
|---|---|---|---|
| Goldschmidt et al. [20] | 20 patients<br>20 samples (10 A, 10 V)<br>10 controls (5 A, 5 V) | DNA extraction: MagNA PureNucleic Acid isolation kit (Roche)<br>*Real-time multiplex PCR with universal primers and specific probes* for genera *Staphylococcus*, *Streptococcus*, *Haemophilus*, *Pseudomonas*, *Enterobacteria*, *Acinetobacter*<br>*16S rDNA primers*<br>For *Corynebacterium* and *Propionibacterium*, original sets of primers and probes were designed | Culture +: 75 % (15/20)<br>In A: 80 % (8/10) including 1 yeast<br>In V: 70 % (7/10) including 1 *Aspergillus*<br>16S rDNA PCR +: 95 % (19/20)<br>In A: 100 % (10/10)<br>In V: 90 % (9/10)<br>Correlation with culture-positive results in all cases except one: culture positive for yeast and PCR positive for *Enterobacteria*<br>Sensitivity of real-time PCR 0.01 CFU of Bac/µl<br>Control samples were negative |
| Sugita et al. [11] | 19 patients<br>10 postoperative (6 acute, 4 delayed), 1 post-traumatic, 5 endogenous, 2 keratitis, 1 post-intravitreal injection endophthalmitis<br>19 samples (8 A, 11 V)<br>15 controls | DNA extraction: DNA mini kit (Qiagen)<br>*Quantitative real-time PCR assay (TaqMan technology)*<br>*16S rDNA primers*<br>*PCR sequencing in samples with high amounts of DNA (n=9/19)*<br>*16S rDNA primers* | Culture +: 53 % (10/19)<br>In A: 50 % (4/8); in V : 55 % (6/11)<br>16S rDNA PCR +: 95 % (18/19)<br>In A: 88 % (7/8); in V: 100 % (11/11)<br>Correlation with culture: 100 % for positive results<br>Detection limit of the TaqMan RT-PCR for detection of *S. aureus* genome: 10 copies<br>Control samples were negative |
| Bispo et al. [24] | 14 patients<br>12 postoperative (11 acute, 1 acute delayed onset); 2 endogenous endophthalmitis<br>21 samples (10 A, 11 V)<br>62 controls (50 A, 12 V) | DNA extraction: QIAamp DNA mini kit (Qiagen)<br>*Real-time SYBR Green PCR followed by sequencing*<br>*16S rDNA primers*<br>*Multiplex real-time PCR with one gram + specific TaqMan probe and one gram − specific TaqMan probe* | Culture +: 47.6 % (10/21)<br>In A: 40 % (4/10); in V: 54.5 % (6/11)<br>16S rDNA PCR +: 95.3 % (20/21) including 6 mixed sequences<br>In A: 100 % (10/10); in V: 90.9 % (10/11)<br>Four samples showed a mixed amplification signal for gram classification<br>Correlation with culture: 100 % for positive results<br>Detection threshold for *S. epidermidis* was 100 fg/µl with pan-bacterial PCR and 1 pg/µl with gram + specific PCR<br>16S rDNA PCR false-positive rate in controls: 3.2 % (4 % in A, 0 % in V) |

(continued)

**Table 4.1** (continued)

| Study | Population studied, samples/ infection category | Technique | Results |
|---|---|---|---|
| Melo et al. [27] | 11 patients with acute postoperative endophthalmitis<br>9 V, 7 A<br>12 control V, 50 control A | *SYBR Green 16S rDNA-based universal PCR*<br>Gram discrimination by a *multiplex Gram-specific TaqMan-based PCR* | Positivity of real-time PCR in 91 %<br>The cycle threshold cutoff value was 36 for universal PCR (sensitivity 94 %, specificity 100 %) and 38 for Gram-specific PCR (Se 94 %, Sp 100 %)<br>Good correlation between Gram stain, culture, and multiplex PCR for Gram classification |
| Joseph et al. [23] | 64 patients<br>Acute and delayed-onset post-cataract endophthalmitis<br>64 samples (V only)<br>50 negative controls (50 V) | DNA extraction: QIAamp DNA mini kit (Qiagen)<br>*Quantitative real-time PCR with TaqMan probe and sequencing 16S rDNA primers* | Culture +: 34 % (19/64)<br>16S rDNA PCR +: 66 % (37/64) (number of copies detected ranging from $1.42 \times 10^5$ to $3.64 \times 10^7$ copies/ml)<br>Correlation with culture: 100 % for positive results<br>Samples from control cases were negative |
| Aarthi et al. [28] | 35 patients<br>35 samples (19 V, 16 A)<br>26 postoperative (24 acute, 2 chronic), 5 post-trauma, 3 endogenous, 1 panophthalmitis endophthalmitis | DNA extraction using Qiagen DNA mini kit<br>Reverse transcriptase PCR targeting the 16S rRNA region of eubacterial genome<br>dHPLC-based DNA sequencing | Presence of 2 bacterial genomes in 22 (63 %) specimens<br>RT-PCR + 82.8 %: A (68.7 %) and V (94.7 %) |
| Cornut et al. [39] | 17 eyes with post-traumatic endophthalmitis, 19 samples (12 A, 9 V) | DNA extraction with Qiagen kit (Qiagen)<br>*PCR sequencing 16S rDNA primers* | Culture +: 10 % A, 43 % V<br>PCR positive in 22 % A, 50 % V<br>The PCR performed in 16 patients (94 %) was positive in 62 % of the cases and was necessary for 5 who had negative cultures (29 %)<br>Bacterial identification was obtained in 77 % of the cases |

| | | | |
|---|---|---|---|
| Sugita et al. [26] | 26 bacterial endophthalmitis<br>9 fungal endophthalmitis | DNA extraction using a DNA mini kit (Qiagen)<br>*Broad-range PCR* using the AmpliTaq Gold<br>Real-time PCR 7300 system or the LightCycler 480 II instrument<br>*Primers for 16S rDNA, fungal 18S, or 28S rDNA* | 16S PCR positive in 64 % of bacterial endophthalmitis cases<br>18S/28S fungal PCR positive in 61 % of cases |
| Bharathi et al. [40] | 66 endophthalmitis:<br>66 V in<br>Postoperative (33), post-traumatic (18), endogenous (3) endophthalmitis | DNA extraction using a QIAamp DNA mini kit (Qiagen)<br>*Uniplex, nested, semi-nested, multiplex, and nested multiplex PCRs*<br>Primers for 16S rDNA, *P. acnes*, fungal 18S, or 28S rDNA | V: cultures + (24 %), PCR+ (65 %)<br>15 % *P. acnes* genome<br>Nested PCRs (sensitivity 64 %) are greater than uniplex (56 %) and multiplex PCR (55 %). The increase in sensitivity may be attributed to the two amplification cycles<br>100 % similarities between culture and PCR results<br>53 % of the 50 culture-negative specimens showed positive amplification<br>PCR is sufficient for the diagnosis of 54 % of culture-negative cases |
| Brillat-Zaratzian et al. [9] | 23 eyes with bleb-related endophthalmitis | DNA extraction with Qiagen kit (Qiagen)<br>*PCR sequencing*<br>*16S rDNA primers* | 13 patients had A sampled (culture positive in 45 %, pan-bacterial PCR positive in 70 %, bacterial identification in 70 %)<br>10 patients had V sampled (culture positive in 40 %, pan-bacterial PCR positive in 46 %, bacterial identification in 61 %)<br>By combining results of culture and pan-bacterial PCR, a bacterial species was identified in 73.9 %<br>PCR identified causative microorganism in three-quarters of cases, i.e., 21 % more cases than through culture alone |

*A* aqueous, *V* vitreous, *PCR* polymerase chain reaction, *PPV* pars plana vitrectomy, *RFLP* restriction fragment length polymorphism

Recently, a *reverse transcriptase PCR* [28] was evaluated in 35 endophthalmitis cases with PCR positivity in 38 % of the aqueous samples and 95 % of the vitreous samples. Selecting 16S rRNA as a target gene had several advantages: the 16S rRNA is essential for the viability of all bacteria and is a multicopy gene with a longer half-life as compared to mRNA.

**Multiplex PCR** requires only 2–3 h and can screen rapidly for the presence of a large number of infectious antigens [20, 26]. This real-time PCR may also be used to measure the DNA load. Acute endophthalmitis is usually associated with a high number of bacterial DNA copies [26].

**Specific PCR Techniques** are rarely used as a first-line diagnostic test in endophthalmitis patients [20, 23, 24, 26]. As compared to pan-bacterial PCR, specific PCR tests allow faster (1–3 h) and more sensitive detection of target bacterial species. Goldschmidt et al. [20] reported the use of PCR tests targeting bacterial species belonging to the same bacterial family or genus (*Enterobacteriaceae*, *Propionibacteriaceae*, *Staphylococcus*, *Streptococcus*, *Haemophilus*, *Pseudomonas*, *Acinetobacter*, *Corynebacterium*). Bispo et al. [24] published a series of 14 patients using real-time PCR incorporating marked nucleotides followed by sequencing, also with a 95 % identification rate versus 47.6 % in culture. However, the sequencing could not be interpreted in an appreciable number of cases in this series. Joseph et al. [23] reported a large series of 64 patients, demonstrating the quantitative value of a real-time PCR method, but with lower identification rates: 66 % in PCR and 34 % in culture. These real-time techniques appear to be more sensitive and more rapid than conventional techniques (the amplification and detection procedures are carried out simultaneously in the same tube).

The development of DNA chips, also called DNA microarrays or biochips, i.e., collecting many specific hybridization probes on the same medium, is currently being studied. *DNA microarray technology* allows simultaneous identification of a wide variety of genes, rapid determination of the genetic profile of a microorganism, and parallel identification of different microorganisms in a single assay. This technique has recently been applied to the vitreous specimens of patients infected with *Klebsiella pneumoniae*, *Streptococcus agalactiae*, and *Candida parapsilosis* [29, 42].

**Quantitative Real-Time PCR** The ability to collect quantitative information on bacterial infections in the eye should be useful in helping determine clinical diagnoses and therapeutic follow-ups [11, 26, 27].

### *4.1.4 Contribution of PCR to the Diagnosis of Post-traumatic Endophthalmitis*

Endophthalmitis occurs at a higher frequency following eye trauma than after eye surgery, and post-traumatic endophthalmitis occurs in approximately 7 % of patients

with penetrating eye injuries [43]. *Staphylococcus epidermidis* has been implicated in 22–42 % of these cases, followed by *Bacillus* (11–29 %), *Streptococcus* (11–14 %), and gram-negative bacteria (10–22 %) [43–46]. Gram-negative bacteria are more commonly associated with post-traumatic endophthalmitis cases with an intraocular foreign body.

Mixed infections are significantly more frequent in this context (11–30 %) [45]. The use of denaturing high performance liquid chromatography-based identification of the bacterial genome may be useful since the presence of mixed genomes can be identified separately and easily [28].

Fungal infections account for 5–15 % of cases of post-traumatic endophthalmitis, particularly cases of wound contamination by plant material [47]. In this context, it can be useful to use broad-range real-time PCR for fungi, measuring the amplification of the target fungal 28S rRNA gene or the *Candida* or *Aspergillus* 18S rRNA genes [8, 22]. This latter study [8] showed PCR-positive samples all had significantly high numbers of copies of *Candida*, *Aspergillus*, or *Cryptococcus* DNA.

In a recent series [41], we showed that the pan-bacterial PCR was positive in 62 % of cases and was indispensable to the microbiological diagnosis for five patients who had negative cultures (29 %). Finally, bacterial identification was obtained in 77 % of cases, most of the time gram-positive bacteria. Pan-bacterial PCR is also useful to test for *P. acnes*, which was detected in up to 17 % of patients in one series [43].

### *4.1.5 Contribution of PCR to the Diagnosis of Fungal Endophthalmitis*

The overall incidence of fungal endophthalmitis is low (3–8 % of endophthalmitis cases). The incidence is 13–20 %, however, in areas with tropical climates, such as in Southern Florida [44, 48] and India [45, 49]. Universal primers complementary to a conserved sequence of either the 18S rRNA gene [10, 50] or the 28S rRNA gene [51] common to all fungi have been used with intraocular specimens. Sensitivity has been found higher in vitreous samples than in aqueous humor samples [51].

Other molecular techniques for fungal identification have been reported such as the use of specific nested PCR [52] or semi-nested PCR targeting the internal transcribed spacer region, a multicopy gene (used in molecular taxonomy to determine the species level) [53–55].

More recently, broad-range (18S rRNA sequences) quantitative real-time PCR has been developed and evaluated in patients with endogenous or post-traumatic endophthalmitis ($n=7$) [22]. This technique allowed rapid identification of fungal DNA and quantification of fungal copies for *Candida* and *Aspergillus* DNA.

All these studies suggest that PCR is a more sensitive and rapid diagnostic tool compared with conventional cultures. However, these studies included a limited number of patients, and the sensitivity of PCR techniques should be further analyzed.

## 4.2 Conclusion

To optimize the detection of microorganisms causing endophthalmitis, it is preferable to obtain an early collection of vitreous and to apply both conventional culture and molecular biology techniques (pan-bacterial PCR or real-time PCR), since the two approaches are complementary. For samples collected at the time of vitrectomy, pan-bacterial PCR performed on diluted vitreous is as useful as on undiluted vitreous. PCR-based techniques are more sensitive than culture for the detection and identification of fastidious bacteria (e.g., *Granulicatella*, *Moraxella*, *P. acnes*, and *Mycobacterium* species) and when patients have received an intravitreal antibiotic before the collection of intraocular samples. Recent molecular techniques allow rapid and specific microbiological diagnosis, can screen rapidly for the presence of a large number of infectious antigens, and quantify bacterial loads.

**Conflict of Interest** The authors declare that they have no conflict of interest.

## References

1. Kresloff MS, Castellarin AA, Zarbin MA. Endophthalmitis. Surv Ophthalmol. 1998;43:193–224.
2. Chiquet C, Maurin M, Thuret G, et al., French Institutional Endophthalmitis Study (FRIENDS) Group. Analysis of diluted vitreous samples from vitrectomy is useful in eyes with severe acute postoperative endophthalmitis. Ophthalmology. 2009;116:2437–41.e1. doi:10.1016/j.ophtha.2009.06.007.
3. Okhravi N, Adamson P, Lightman S. Use of PCR in endophthalmitis. Ocul Immunol Inflamm. 2000;8:189–200.
4. Chiquet C, Cornut P-L, Benito Y, et al., French Institutional Endophthalmitis Study Group. Eubacterial PCR for bacterial detection and identification in 100 acute postcataract surgery endophthalmitis. Invest Ophthalmol Vis Sci. 2008;49:1971–78. doi:10.1167/iovs.07-1377.
5. Cornut P-L, Chiquet C, Bron A, et al., FRIENDS Group. Microbiologic identification of bleb-related delayed-onset endophthalmitis caused by moraxella species. J Glaucoma. 2008;17:541–5. doi:10.1097/IJG.0b013e31816299ec.
6. Namdari H, Kintner K, Jackson BA, et al. Abiotrophia species as a cause of endophthalmitis following cataract extraction. J Clin Microbiol. 1999;37:1564–6.
7. Lee AY, Akileswaran L, Tibbetts MD, et al. Identification of torque teno virus in culture- negative endophthalmitis by representational deep DNA sequencing. Ophthalmology. 2015;122:524–30.
8. Ogawa M, Sugita S, Watanabe K, et al. Novel diagnosis of fungal endophthalmitis by broad-range real-time PCR detection of fungal 28S ribosomal DNA. Graefes Arch Clin Exp Ophthalmol Albrecht Von Graefes Arch Für Klin Exp Ophthalmol. 2012;250:1877–83. doi:10.1007/s00417-012-2015-7.
9. Brillat-Zaratzian E, Bron A, Aptel F, et al. FRIENDS Group: clinical and microbiological characteristics of post-filtering surgery endophthalmitis. Graefes Arch Clin Exp Ophthalmol Albrecht Von Graefes Arch Für Klin Exp Ophthalmol. 2014;252:101–7. doi:10.1007/s00417-013-2503-4.
10. Lohmann CP, Heeb M, Linde HJ, et al. Diagnosis of infectious endophthalmitis after cataract surgery by polymerase chain reaction. J Cataract Refract Surg. 1998;24:821–6.

11. Sugita S, Shimizu N, Watanabe K, et al. Diagnosis of bacterial endophthalmitis by broad-range quantitative PCR. Br J Ophthalmol. 2011;95:345–9. doi:10.1136/bjo.2009.171504.
12. Chiquet C, Lina G, Benito Y, et al. Polymerase chain reaction identification in aqueous humor of patients with postoperative endophthalmitis. J Cataract Refract Surg. 2007;33:635–41. doi:10.1016/j.jcrs.2006.12.017.
13. Knox CM, Cevallos V, Margolis TP, Dean D. Identification of bacterial pathogens in patients with endophthalmitis by 16S ribosomal DNA typing. Am J Ophthalmol. 1999;128:511–2.
14. Lohmann CP, Linde HJ, Reischl U. Improved detection of microorganisms by polymerase chain reaction in delayed endophthalmitis after cataract surgery. Ophthalmology. 2000;107:1047–51; discussion 1051–52.
15. Okhravi N, Adamson P, Carroll N, et al. PCR-based evidence of bacterial involvement in eyes with suspected intraocular infection. Invest Ophthalmol Vis Sci. 2000;41:3474–9.
16. Thompson CC, Chimetto L, Edwards RA, et al. Microbial genomic taxonomy. BMC Genomics. 2013;14:913. doi:10.1186/1471-2164-14-913.
17. Devulder G, Perrière G, Baty F, Flandrois JP. BIBI, a bioinformatics bacterial identification tool. J Clin Microbiol. 2003;41:1785–7.
18. Anand AR, Madhavan HN, Therese KL. Use of polymerase chain reaction (PCR) and DNA probe hybridization to determine the Gram reaction of the infecting bacterium in the intraocular fluids of patients with endophthalmitis. J Infect. 2000;41:221–6. doi:10.1053/jinf.2000.0731.
19. Carroll NM, Jaeger EE, Choudhury S, et al. Detection of and discrimination between gram-positive and gram-negative bacteria in intraocular samples by using nested PCR. J Clin Microbiol. 2000;38:1753–7.
20. Goldschmidt P, Degorge S, Benallaoua D, et al. New test for the diagnosis of bacterial endophthalmitis. Br J Ophthalmol. 2009;93:1089–95. doi:10.1136/bjo.2008.152181.
21. Fenollar F, Raoult D. Molecular genetic methods for the diagnosis of fastidious microorganisms. APMIS Acta Pathol Microbiol Immunol Scand. 2004;112:785–807. doi:10.1111/j.1600-0463.2004.apm11211-1206.x.
22. Sugita S, Kamoi K, Ogawa M, et al. Detection of Candida and Aspergillus species DNA using broad-range real-time PCR for fungal endophthalmitis. Graefes Arch Clin Exp Ophthalmol Albrecht Von Graefes Arch Für Klin Exp Ophthalmol. 2012;250:391–8. doi:10.1007/s00417-011-1819-1.
23. Joseph CR, Lalitha P, Sivaraman KR, et al. Real-time polymerase chain reaction in the diagnosis of acute postoperative endophthalmitis. Am J Ophthalmol. 2012;153:1031–1037.e2. doi:10.1016/j.ajo.2011.12.007.
24. Bispo PJ, de Melo GB, Hofling-Lima AL, Pignatari AC. Detection and gram discrimination of bacterial pathogens from aqueous and vitreous humor using real-time PCR assays. Invest Ophthalmol Vis Sci. 2011;52:873–81. doi:10.1167/iovs.10-5712.
25. Therese KL, Anand AR, Madhavan HN. Polymerase chain reaction in the diagnosis of bacterial endophthalmitis. Br J Ophthalmol. 1998;82:1078–82.
26. Sugita S, Ogawa M, Shimizu N, et al. Use of a comprehensive polymerase chain reaction system for diagnosis of ocular infectious diseases. Ophthalmology. 2013;120:1761–8. doi:10.1016/j.ophtha.2013.02.020.
27. Melo GB, Bispo PJ, Campos Pignatari AC, Höfling-Lima AL. Real-time polymerase chain reaction test to discriminate between contamination and intraocular infection after cataract surgery. J Cataract Refract Surg. 2011;37:1244–50. doi:10.1016/j.jcrs.2011.01.025.
28. Aarthi P, Bagyalakshmi R, Therese KL, et al. Optimization and application of a reverse transcriptase polymerase chain reaction to determine the bacterial viability in infectious endophthalmitis. Curr Eye Res. 2012;37:1114–20. doi:10.3109/02713683.2012.704476.
29. Sakai T, Kohzaki K, Watanabe A, et al. Use of DNA microarray analysis in diagnosis of bacterial and fungal endophthalmitis. Clin Ophthalmol Auckl NZ. 2012;6:321–6. doi:10.2147/OPTH.S29230.
30. Fisch A, Salvanet A, Prazuck T, et al. Epidemiology of infective endophthalmitis in France. The French Collaborative Study Group on Endophthalmitis. Lancet Lond Engl. 1991;338:1373–6.

31. Anonymous. Microbiologic factors and visual outcome in the endophthalmitis vitrectomy study. Am J Ophthalmol. 1996;122:830–46.
32. Han DP, Wisniewski SR, Kelsey SF, et al. Microbiologic yields and complication rates of vitreous needle aspiration versus mechanized vitreous biopsy in the Endophthalmitis Vitrectomy Study. Retina Phila Pa. 1999;19:98–102.
33. Han DP, Wisniewski SR, Wilson LA, et al. Spectrum and susceptibilities of microbiologic isolates in the Endophthalmitis Vitrectomy Study. Am J Ophthalmol. 1996;122:1–17.
34. Hassan IJ, MacGowan AP, Cook SD. Endophthalmitis at the Bristol Eye Hospital: an 11-year review of 47 patients. J Hosp Infect. 1992;22:271–8.
35. Schrader W. Endogenous candida endophthalmitis. Early diagnosis and early vitrectomy improve the prognosis. Fortschritte Ophthalmol Z Dtsch Ophthalmol Ges. 1990;87:331–5.
36. Hykin PG, Tobal K, McIntyre G, et al. The diagnosis of delayed post-operative endophthalmitis by polymerase chain reaction of bacterial DNA in vitreous samples. J Med Microbiol. 1994;40:408–15.
37. Bagyalakshmi R, Madhavan HN, Therese KL. Development and application of multiplex polymerase chain reaction for the etiological diagnosis of infectious endophthalmitis. J Postgrad Med. 2006;52:179–82.
38. Sowmya P, Madhavan HN. Diagnostic utility of polymerase chain reaction on intraocular specimens to establish the etiology of infectious endophthalmitis. Eur J Ophthalmol. 2009;19:812–7.
39. Cornut P-L, Thuret G, Creuzot-Garcher C, et al., French Institutional Endophthalmitis Study Group. Relationship between baseline clinical data and microbiologic spectrum in 100 patients with acute postcataract endophthalmitis. Retina Phila Pa. 2012;32:549–57. doi:10.1097/IAE.0b013e3182205996.
40. Bharathi MJ, Rameshkumar G, Ramakrishnan R, et al. Comparative evaluation of uniplex, nested, semi-nested, multiplex and nested multiplex PCR methods in the identification of microbial etiology of clinically suspected infectious endophthalmitis. Curr Eye Res. 2013;38:550–62. doi:10.3109/02713683.2013.772205.
41. Cornut P-L, Boisset S, Romanet J-P, et al. Principles and applications of molecular biology techniques for the microbiological diagnosis of acute post-operative endophthalmitis. Surv Ophthalmol. 2013;59(3):286–303. doi:10.1016/j.survophthal.2013.08.002.
42. Sakai H, Procop GW, Kobayashi N, et al. Simultaneous detection of Staphylococcus aureus and coagulase-negative staphylococci in positive blood cultures by real-time PCR with two fluorescence resonance energy transfer probe sets. J Clin Microbiol. 2004;42:5739–44. doi:10.1128/JCM.42.12.5739-5744.2004.
43. Essex RW, Yi Q, Charles PG, Allen PJ. Post-traumatic endophthalmitis. Ophthalmology. 2004;111:2015–22. doi:10.1016/j.ophtha.2003.09.041.
44. Affeldt JC, Flynn HW, Forster RK, et al. Microbial endophthalmitis resulting from ocular trauma. Ophthalmology. 1987;94:407–13.
45. Kunimoto DY, Das T, Sharma S, et al. Microbiologic spectrum and susceptibility of isolates: part II. Posttraumatic endophthalmitis. Endophthalmitis Research Group. Am J Ophthalmol. 1999;128:242–4.
46. Peyman GA, Carroll CP, Raichand M. Prevention and management of traumatic endophthalmitis. Ophthalmology. 1980;87:320–4.
47. Gupta A, Srinivasan R, Kaliaperumal S, Saha I. Post-traumatic fungal endophthalmitis–a prospective study. Eye Lond Engl. 2008;22:13–7. doi:10.1038/sj.eye.6702463.
48. Forster RK, Abbott RL, Gelender H. Management of infectious endophthalmitis. Ophthalmology. 1980;87:313–9.
49. Anand AR, Therese KL, Madhavan HN. Spectrum of aetiological agents of postoperative endophthalmitis and antibiotic susceptibility of bacterial isolates. Indian J Ophthalmol. 2000;48:123–8.
50. Jaeger EE, Carroll NM, Choudhury S, et al. Rapid detection and identification of Candida, Aspergillus, and Fusarium species in ocular samples using nested PCR. J Clin Microbiol. 2000;38:2902–8.

51. Anand A, Madhavan H, Neelam V, Lily T. Use of polymerase chain reaction in the diagnosis of fungal endophthalmitis. Ophthalmology. 2001;108:326–30.
52. Hidalgo JA, Alangaden GJ, Eliott D, et al. Fungal endophthalmitis diagnosis by detection of Candida albicans DNA in intraocular fluid by use of a species-specific polymerase chain reaction assay. J Infect Dis. 2000;181:1198–201. doi:10.1086/315333.
53. Bagyalakshmi R, Therese KL, Madhavan HN. Application of semi-nested polymerase chain reaction targeting internal transcribed spacer region for rapid detection of panfungal genome directly from ocular specimens. Indian J Ophthalmol. 2007;55:261–5.
54. Ferrer C, Alio J, Rodriguez A, et al. Endophthalmitis caused by Fusarium proliferatum. J Clin Microbiol. 2005;43:5372–5. doi:10.1128/JCM.43.10.5372-5375.2005.
55. Tarai B, Gupta A, Ray P, et al. Polymerase chain reaction for early diagnosis of post-operative fungal endophthalmitis. Indian J Med Res. 2006;123:671–8.