

Microbiologic Diagnosis of Acute Endophthalmitis

BY CHRISTOPHE CHIQUET, MD, PhD

Endophthalmitis is most commonly caused by exogenous sources. Exogenous bacterial endophthalmitis is a known postoperative complication of elective ocular surgery and after perforating ocular trauma. The severity of this type of infection and the speed with which lesions evolve requires rapid diagnostic confirmation, which, in turn, influences the therapeutic management of the patient and hence the anatomic and functional prognosis. Molecular biology techniques that can rapidly detect and identify causative microorganisms have improved the ability to correctly identify the microbiologic etiology of endophthalmitis.

SAMPLING TECHNIQUES

Samples for microbiologic evaluation can be collected from the aqueous humor (AH) or vitreous humor (VH). Ideally, a sample is collected in the OR after local antisepsis, under local or general anesthesia, and before any intravitreal administration of antibiotics.

VH samples tend to yield more accurate results; they may be collected via transscleral puncture with a needle through the pars plana (Figure 1A) or via posterior vitrectomy performed for diagnostic and/or therapeutic purposes (Figure 1B). About 200 to 300 μ L of sample can be collected by means of a vitreous puncture using a 25-gauge needle attached to a 2- or 3-cc syringe.

In severe endophthalmitis, the density of intravitreal suppuration may preclude needle aspiration of the VH; in such cases, pure or diluted VH can be collected via a pars plana vitrectomy in the following manner: After connecting a 3-cc syringe to the aspiration line of the vitreotome and flushing the vitreotome tubing with air (to prevent dilution of the sample with liquid in the tubing), the surgical assistant aspirates the vitreous gel without perfusion of infusion fluid. Then the vitrectomy can be continued while infusion fluid is injected into the posterior chamber to restore intraocular pressure. The

mixture of VH and infusion fluid collected is referred to as *diluted VH*.

Panbacterial polymerase chain reaction (PCR: ie, 16S rRNA gene amplification and sequencing; more on this below) appears to have comparable sensitivity with pure and diluted VH.¹ Collecting diluted VH is easier and induces less intraocular hypotonia, thereby limiting the risk of choroidal hematoma, retinal detachment, and displacement of the infusion terminal. The contents of the vitrectomy cassette can also be cultured. Microbiologic tests performed on both pure VH and the vitrectomy cassette yield a diagnosis in about 57% of cases, whereas with the pure VH or the vitrectomy cassette alone the rates are 44% and 49%, respectively.^{2,3}

If one opts to sample the vitrectomy cassette, caution must be taken to avoid exogenous contamination of the sample. A high vitreotome cutting speed (up to 1500 cuts per minute [cpm]) does not affect the result of bacteriologic cultures.⁴ Transconjunctival vitrectomy systems may be useful, notably in patients with glaucoma, but the need for scleral tunneling in patients with

At a Glance

- Microbiologic diagnosis of endophthalmitis may have implications for selecting appropriate therapy and thus may influence visual and anatomic outcomes.
- New methods of polymerase chain reaction (PCR) analysis are making this diagnostic modality more readily applicable in this setting.
- Panbacterial PCR may be important for microbiologic diagnosis and may be complementary to standard cultures.

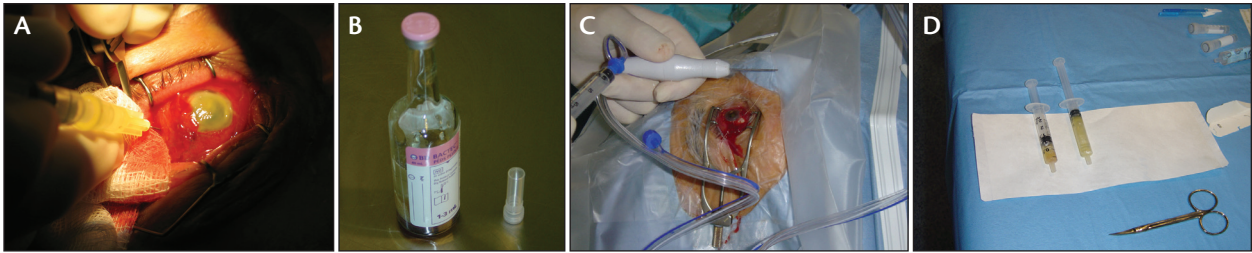


Figure 1. Ocular samples during endophthalmitis: vitreous needle puncture (A), sample taken during pars plana vitrectomy (B), pediatric hemoculture vials and microtube for PCR (C), and aspect of vitreous infected in 2.5-ml syringes during a vitrectomy (D).

chemosis may be a limiting factor.⁵ Alternatively, the endoscope is useful in cases of severe corneal opacity.⁶

The AH is technically easier to collect than the VH, this can be done through a transcorneal puncture of the anterior chamber using a 25-, 27- or 30-gauge needle for aspiration, and this may translate to a lower risk of inducing iatrogenic complications. On the other hand, because the AH is rapidly renewed by the body, it may have lower diagnostic value compared with VH. If the AH is sampled, a volume of 100 to 200 μ L of AH should be collected, with half the sample reserved for culture and the other half for molecular diagnosis with PCR. AH collection may not be feasible if there is intraocular pus formation.

Conjunctival samples usually have no diagnostic value because they typically represent patients' commensal flora. However, collecting surface and intraocular samples may be useful in the presence of a infected filtration bleb, keratitis, or suture abscess. The posterior capsule and, less commonly, the intraocular implant can be analyzed in patients with chronic endophthalmitis.

Packaging, Preservation, and Delivery of Samples

It is preferable to inject intraocular samples into liquid culture media (blood-culture bottle or brain-heart infusion [BHI] broth tube; Figure 1C) in the OR and under sterile conditions. Any sample intended for PCR analysis should be placed in a sterile screw-capped DNA-free tube. The minimum volume for molecular analysis is approximately 50 μ L. The tube should be placed in a secondary sterile container. The time it takes to deliver the sample to the microbiology laboratory should not exceed 2 hours. If these conditions cannot be achieved, the inoculated cultures (blood culture bottles and BHI broth tubes) must be stored at 37°C and PCR tubes at 4°C for a maximum of 48 hours or at -20°C for longer periods.

BACTERIOLOGIC IDENTIFICATION TECHNIQUES

Culture Methods

The isolation of microorganisms in culture is the reference method for determining the etiology of



Figure 2. Colonies of *S. aureus* (left) and *S. epidermidis* (right) on Columbia gelose with sheep blood.

endophthalmitis. If the sample volume is sufficient (Figure 1D), approximately 20 μ L is spread on each of two sterile slides for direct examination. One slide is stained with May-Grünwald Giemsa (MGG) stain to identify leukocytes and fungi, and the second slide is stained with Gram stain to identify bacteria and fungi. Direct microscopic examination a few minutes after collection of samples may reveal the presence of leukocytes and, occasionally, bacteria, yeast, or hyphae. The diagnosis of intraocular infection can be confirmed by direct examination in about 19% of cases. Direct examination of intraocular samples is most often negative (81% of cases) both because of the small volume of sample collected and a low intraocular bacterial inoculum.⁷

In the clinical microbiology laboratory, a number of culture media are used to allow isolation of the microorganisms most frequently involved in exogenous endophthalmitis, including BHI broth, thioglycollate broth, blood agar, chocolate agar, MacConkey agar, and Sabouraud agar (Figure 2). Intraocular samples can also be inoculated to blood culture bottles in the OR, preferably a pediatric bottle to maintain an optimal sample: culture medium ratio. This technique has several advantages over conventional culture media, including higher sensitivity (owing to permanent agitation of cultures) and a lower risk of contamination during sample handling. These blood culture media allow the isolation of aerobic bacteria (ie, staphylococci, streptococci,

enterobacteria, etc.), preferential anaerobic bacteria (eg, *Propionibacterium acnes*), and yeasts (eg, *Candida* spp.).

Cultures should be immediately stored at 37°C or at ambient temperature for some filamentous fungi. They must be maintained for at least 2 weeks before being considered sterile. Species identification and an antibiogram (or an antifungigram) can be performed only after microbial growth is detected.

Negative culture results of AH and/or VH samples may be related to the small sample volume; the presence of bacteria on solid surfaces (eg, intraocular implant, lens capsule, remaining crystalline masses, bacteria coated with a biofilm, fibrin, and polysaccharides); the use of antibiotics before sample harvesting (notably antibiotic treatment); fastidious growth of certain bacteria such as *P. acnes* (slow growth) or *Granulicatella* spp. (indispensable growth factors).

MOLECULAR BIOLOGY TECHNIQUES

PCR analysis is used to amplify the DNA of microorganisms in clinical samples. It has been applied over the past few years in etiologic diagnosis of endophthalmitis, and its utility has recently been enhanced with the introduction of real-time PCR technology. The DNA of any bacterium can be amplified by targeting the gene coding 16S rRNA, or any mycete can be amplified by targeting the 18S rRNA. This approach is called *universal amplification* or *panbacterial PCR* when the gene coding for 16S rRNA is amplified.^{1,8-11} Panbacterial PCR provides a plausible diagnostic approach when there is no prior knowledge of the bacterial species involved. The different PCR techniques have been summarized in a recent review.¹² Recently, PCRs targeting particular pathogens have been developed.

Specific PCRs are more sensitive and more rapid than panbacterial PCR. There are advantages and disadvantages to the use of specific PCRs: On the one hand, because they are specific to a given genus or species, there is a lower risk of nonspecific amplifications; on the other, they require a directed diagnosis (*a priori* search for a bacterium), and, thus, the inciting microorganism may be missed. Several techniques have been described and validated: multiplex PCR (allowing a limited number of genes to be analyzed in one reaction), real-time PCR,^{13,14} quantitative real-time PCR (with high numbers of bacterial DNA copies in cases of endophthalmitis),¹⁵⁻¹⁷ and reverse transcriptase PCR (to determine the viability of bacteria).¹⁸ Another promising method for identifying microorganisms in endophthalmitis, *DNA microarray analysis*, is a genotyping method that allows simultaneous identification of a wide variety of genes and rapid determination of the genetic profile of a microorganism.¹⁹

It is important to note that a negative PCR result does not exclude the diagnosis of endophthalmitis because the

sensitivity of these techniques never reaches 100%. PCR techniques are nonetheless more sensitive and more rapid than culturing, particularly for slow-growing and fastidious microorganisms.

In all settings, rigorous surgical asepsis should be adhered to, so as to prevent contamination of the sample. Samples should be transferred to a sterile DNA-free test tube. Laboratory contaminations must also be prevented. In our experience, adhering to a strict methodology has reduced the false-positive rate of panbacterial PCR to between zero and 2%.

BACTERIOLOGIC RESULTS

Posttraumatic Endophthalmitis

Posttraumatic endophthalmitis represents about 6.8% of all cases.²⁰ *Staphylococcus epidermidis* is the most common cause (in approximately 22% to 42% of cases), followed by *Bacillus* spp. (11% to 29%), streptococci (11% to 14%), and Gram-negative bacteria (10% to 22%).²⁰⁻²⁵ *P. acnes* may also be an important cause, as it was detected in 17% of patients in one series.²⁰ In a recent series, we found that panbacterial PCR was positive in 62% of cases and was indispensable in the microbiologic diagnosis of five patients who had negative cultures (29%).²⁶ We obtained bacterial identification in 77% of cases, and most of the time the causative agents were Gram-positive bacteria.

Mixed infections are frequent in posttraumatic cases (11% to 30% of cases).²⁵ *Bacillus cereus* and *B. licheniformis*—both of which are sporulated Gram-positive bacilli—are considered highly virulent because they produce enterotoxin, phospholipase C, hemolysin, and other proteolytic enzymes. Finally, infections caused by species of the *Clostridium* genus secondary to telluric contamination should not be overlooked.^{24,27}

Endophthalmitis occurs in about 1% to 13% of cases of trauma involving an intraocular foreign body (IOFB).²⁸⁻³⁰ In such cases, *Bacillus* spp. are commonly the inciting microorganisms (36% in the United States), as are staphylococci (45%) and streptococci (5%).³¹ For eyes that have been injured with an IOFB without identification of endophthalmitis, culture of the IOFB is positive in about 28% of cases.²⁸

Postoperative Acute Endophthalmitis

In cases of acute postoperative bacterial endophthalmitis, the ocular sample is positive in 22% to 30% of cases for AH³²⁻³⁵ and 40% to 69% for VH.^{2,3,7,35-37} Gram-positive bacteria clearly predominate: 94.1% in the American EVS^{7,36,38} and 97% in a multicenter study conducted by the French FRIENDS group.⁹ Among Gram-positive bacteria,⁹ *S. epidermidis* is the leading cause (45% to 50%), followed by streptococci (24% to 37.7%) and *S. aureus*

(7.5% to 11.5%). Gram-negative bacteria (eg, *Proteus* spp., *Escherichia coli*, *Pseudomonas*, *Klebsiella*, and *Serratia* spp.) account for 3% to 15% of bacteria isolated in such cases. Infection with two bacteria has been described in several studies, with a highly variable frequency between zero and 29%.³⁹⁻⁴¹ In our experience, coinfection is rare in this category of endophthalmitis.

VALUE OF PANBACTERIAL PCR IN THE DIAGNOSIS OF MICROBIOLOGIC ENDOPTHALMITIS

The advantage of PCR for the microbiologic diagnosis of endophthalmitis was first reported in 1994.⁴² Molecular biology techniques are useful in cases of acute endophthalmitis^{8,9,43-47} and chronic endophthalmitis.^{42,44,46,48} Use of panbacterial PCR in postoperative acute or delayed-onset endophthalmitis associated with conventional bacteriologic culture techniques makes it possible to increase the bacterial identification rate in AH (47%) and the vitreous (68%).^{8,9,34,49}

This technique cannot entirely replace cultures, which can also be used for antibiograms. In our experience, panbacterial PCR is indispensable for microbiologic diagnosis. However, the available evidence shows that panbacterial PCR and standard cultures are complementary in microbiologic identification. After treatment with intravitreal injection of antibiotics, panbacterial PCR alone, performed on pure or diluted vitreous samples, can detect the bacterial agent in 72% of cases and therefore is the gold standard.¹ ■

Christophe Chiquet, MD, PhD, is a professor in the department of ophthalmology, University Hospital of Grenoble, France. Prof. Chiquet may be reached at +33-476768457; fax: +33-476765757; or cchiquet@chu-grenoble.fr.

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