Use of a Comprehensive Polymerase Chain Reaction System for Diagnosis of Ocular Infectious Diseases

Sunao Sugita, MD, PhD,[1,2] Manabu Ogawa, MD, PhD,[1] Norio Shimizu, PhD,[3] Tomohiro Morio, MD, PhD,[4] Nobuyuki Ohguro, MD, PhD,[5] Kei Nakai, MD, PhD,[6] Kazuichi Maruyama, MD, PhD,[7] Kenji Nagata, MD,[7] Atsunobu Takeda, MD, PhD,[8] Yoshihiko Usui, MD, PhD,[9] Koh-Hei Sonoda, MD, PhD,[10] Masaru Takeuchi, MD, PhD,[11] Manabu Mochizuki, MD, PhD[1]

Purpose: To measure the genomic DNA of ocular infectious pathogens in ocular fluids and to analyze the clinical relevance of these pathogens in uveitis and endophthalmitis.

Design: Prospective clinical case series.

Participants: A total of 500 patients with infectious uveitis and endophthalmitis were examined at Tokyo Medical and Dental University, Tokyo Medical University, Kyushu University, Osaka University, and Kyoto Prefectural University, all in Japan.

Methods: Genomic DNA of bacteria, fungi, parasites, and viruses in collected intraocular samples were examined by comprehensive polymerase chain reaction (PCR). Samples were analyzed first by multiplex PCR and quantitative real-time PCR for human herpes viruses (HHVs) 1 through 8 and toxoplasma. Subsequently, samples were examined by broad-range real-time PCR for bacterial 16S and fungal 18S/28S ribosomal DNA (rDNA).

Main Outcome Measures: Infectious uveitis and endophthalmitis diagnoses were obtained when using the PCR system. Calculations of the positivity and the diagnostic parameters such as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) also were evaluated.

Results: In all of the tested infectious uveitis and endophthalmitis patients, either herpes simplex virus type 1 (n = 18), herpes simplex virus type 2 (n = 4), varicella-zoster virus (n = 55), Epstein-Barr virus (n = 17), cytomegalovirus (n = 68), HHV type 6 (n = 2), toxoplasma (n = 6), bacterial 16S (n = 33), or fungal 18S/28S (n = 11) genome was detected. Neither HHV type 7 nor HHV type 8 DNA was detected in any of the samples. Of the 21 false-negative results found during the PCR analyses, 12 cases were negative for patients clinically suspected of having bacterial endophthalmitis. Conversely, false-positive results for the comprehensive PCR examinations occurred in only 3 cases that subsequently were found to have bacterial 16S rDNA. Diagnostic parameters for the sensitivity, specificity, PPV, and NPV of our PCR examinations were 91.3%, 98.8%, 98.6%, and 92.4%, respectively.

Conclusions: Use of our comprehensive PCR assay to examine ocular samples in patients with endophthalmitis and uveitis seems to be clinically useful for detecting infectious antigen DNA. Thus, this PCR method is a reliable tool for both diagnosing ocular disorders and further screening of patients for intraocular infections.

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Infectious uveitis and endophthalmitis are sight-threatening diseases caused by human pathogenic agents. Virus infections, especially herpes viruses, are known to cause ocular inflammations such as retinitis, uveitis, retinal vasculitis, conjunctivitis, corneal endotheliitis, and keratitis. Parasitic infections (e.g., ocular toxoplasmosis) are known to cause intraocular inflammations such as uveitis, whereas bacterial and fungal infections are known to cause endophthalmitis, uveitis, and keratitis. However, when infectious pathogens cause ocular inflammatory disorders, the clinical findings can be very diverse, thereby making the diagnosis of the infection both difficult and time consuming. To ensure that appropriate treatments are performed that will prevent these infectious agents from causing irreversible ocular tissue damage, early examinations that correctly identify the cause of the infections are a necessity.

For more than a decade, diagnostic evaluations of patients with uveitis and endophthalmitis diagnoses have included the use of conventional qualitative polymerase chain reaction (PCR). A PCR diagnosis has proven to be very useful because only a very small sample is required for detection of the infectious agents. However, in the past, the relatively small volume of sample that can be obtained (such as aqueous humor) has made it impossible to conduct comprehensive examinations for ocular inflammatory diseases. Nevertheless, comprehensive diagnoses are needed

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because ocular inflammatory diseases can include various infectious antigens. Additionally, because sudden changes can occur in ocular infectious diseases, it is imperative that patients be diagnosed as early as possible. Therefore, a comprehensive, rapid, and accurate diagnosis using ocular samples is of great importance, and if such a comprehensive PCR system for the diagnosis of ocular infectious disorders could be established, this would be a tremendous help for many clinicians. However, this research tool has not been widely available for use in clinical laboratories. Therefore, this study attempted both to establish novel tests that would be widely available to clinical laboratories and to develop a comprehensive PCR system that could be used to examine bacteria, fungi, parasites, and viruses for the purpose of diagnosing ocular infectious disorders.

Methods

Patients

Intraocular samples of aqueous humor and vitreous fluid were collected from 500 patients with uveitis and endophthalmitis. Underlying pathologic features included endotheliitis, keratouveitis, anterior uveitis or iridocyclitis, acute retinal necrosis (ARN), progressive outer retinal necrosis, cytomegalovirus (CMV) retinitis, human T lymphotropic virus type 1 uveitis, ocular toxoplasmosis, scleritis, Posner-Schlossman syndrome, Fuchs' iridocyclitis, sarcoidosis, Vogt-Koyanagi-Harada disease, Behçet's disease, intraocular lymphoma, idiopathic uveitis, idiopathic retinal vasculitis, bacterial endophthalmitis, and fungal endophthalmitis. At the time of sampling, these patients displayed active intraocular inflammation. In addition, control samples were prepared for this study (n = 100). The control group included noninflammatory diseases such as diabetes, retinal detachment, glaucoma, and others.

An aliquot of 0.1 ml aqueous humor was aspirated with a 30-gauge needle. Nondiluted vitreous fluid samples were collected from uveitis patients who were undergoing vitreous surgery (diagnostic pars plana vitrectomy). Samples were transferred into presterilized microfuge tubes and used for PCR. To ensure that no contamination of the PCR preparation occurred, the DNA amplification and the analysis of the amplified products were carried out in separate laboratories according to a method reported in a previous study.[1]

We consecutively enrolled endophthalmitis and uveitis patients from 2006 through 2010 for a prospective study that was conducted at both our hospital facility (Tokyo Medical and Dental University) and its associated hospitals (Tokyo Medical University, Kyushu University, Osaka University, and Kyoto Prefectural University). After informed consent was obtained from all patients, we collected aqueous humor and vitreous fluid samples. The research followed the tenets of the Declaration of Helsinki and all study protocols were approved by the Institutional Ethics Committees of Tokyo Medical and Dental University and its associated hospitals. The clinical trial was registered on September 7, 2009 (available at: www.umin.ac.jp/ctr/index/-j.htm; study no.: R000002708).

Polymerase Chain Reaction Analyses

The sampling procedure and the PCR methodology are shown in Figure 1. DNA was extracted from the samples using a DNA Mini kit (Qiagen, Valencia, CA) installed on a robotic workstation that was set for automated purification of nucleic acids (BioRobot EZ1 Advanced; Qiagen). Genomic DNA of human herpes virus (HHV), toxoplasma, bacteria, and fungi in the aqueous humor and vitreous fluids was measured through the use of 2 independent PCR assays: (1) a qualitative multiplex PCR that was performed in conjunction with a quantitative real-time PCR, and (2) a broad-range real-time PCR (Fig 1). The multiplex PCR qualitatively measured the genomic DNA of 8 HHVs: herpes simplex virus (HSV) type 1 (HHV-1), HSV-2 (HHV-2), varicella-zoster virus (VZV; HHV-3), Epstein-Barr virus (EBV; HHV-4), CMV (HHV-5), HHV-6, HHV-7, HHV-8, and toxoplasma. The PCR was performed using a LightCycler 480 II instrument (Roche, Basel, Switzerland). If the multiplex PCR results were positive, we then conducted real-time PCR. The real-time PCR was performed using the Amplitaq Gold and the Real-Time PCR 7300 system (Applied Biosystems, Foster City, CA) or the LightCycler 480 II instrument (Roche). Primers and probes for HHV types 1 through 8 and the PCR conditions have been described previously.[2,3] The toxoplasmosis primers and probes also have been reported previously.[4]

To detect DNA for bacterial species (Fig 1), broad-range PCR was performed using the Amplitaq Gold and the Real-Time PCR 7300 system or the LightCycler 480 II instrument in accordance with our previously reported methodology.[1] Primers and probes for the fungal species (fungal 18S or 28S ribosomal DNA [rDNA]) along with the PCR conditions also have been reported previously.[5,6]

Amplification of the human β-globulin gene served as an internal positive extraction and amplification control. The value of the HHV copy number in the sample was considered to be significant when more than 50 copies/ml were observed. Significant differences in the copy number were defined as more than 10 copies/ml for toxoplasmosis, more than 100 copies/ml for bacterial 16S, and more than 10 copies/ml for fungal 18S/28S.

Results

Step 1 of our comprehensive PCR examinations consisted of qualitative multiplex PCR combined with quantitative real-time PCR, whereas step 2 used broad-range real-time PCR (Figs 1 and 2). As seen in the results for a representative positive aqueous humor sample, although we observed a high copy number of bacterial 16S rDNA, the PCR examination indicated that the sample showed negative results for DNA from all other infectious antigens (Fig 2).

In the uveitis and endophthalmitis patients, our comprehensive PCR system results demonstrated positivity in the ocular fluids. As seen in Table 1, multiplex PCR and real-time PCR detected 18 patients with HSV-1 DNA (18 of 500 cases; 3.6% positive). Herpes simplex virus type 2 DNA was detected in only 4 patients (0.8% positive), with all of these patients subsequently diagnosed with ARN. Varicella-zoster virus DNA was detected in the ocular fluid samples of 55 patients (11% positive), whereas EBV DNA was detected in 17 patients (3.4% positive) with various ocular inflammatory disorders. Cytomegalovirus was detected in 68 patients (13.6% positive) with disorders that included corneal endotheliitis, iridocyclitis, and necrotic retinitis. However, our PCR methods detected only 2 HHV-6 DNA cases (0.4% positive), with none of the patients found to have HHV-7 or HHV-8. In addition, toxoplasmosis DNA was detected in only 6 samples (1.2% positive), with all of these patients found to have active uveitis with ocular toxoplasmosis. Overall, our multiplex PCR and real-time PCR analyses identified 170 PCR-positive patients (34% positive; Table 1).

However, when we used broad-range real-time PCR to screen for detection of bacterial 16S and fungal 18S/28S rDNA in infec-

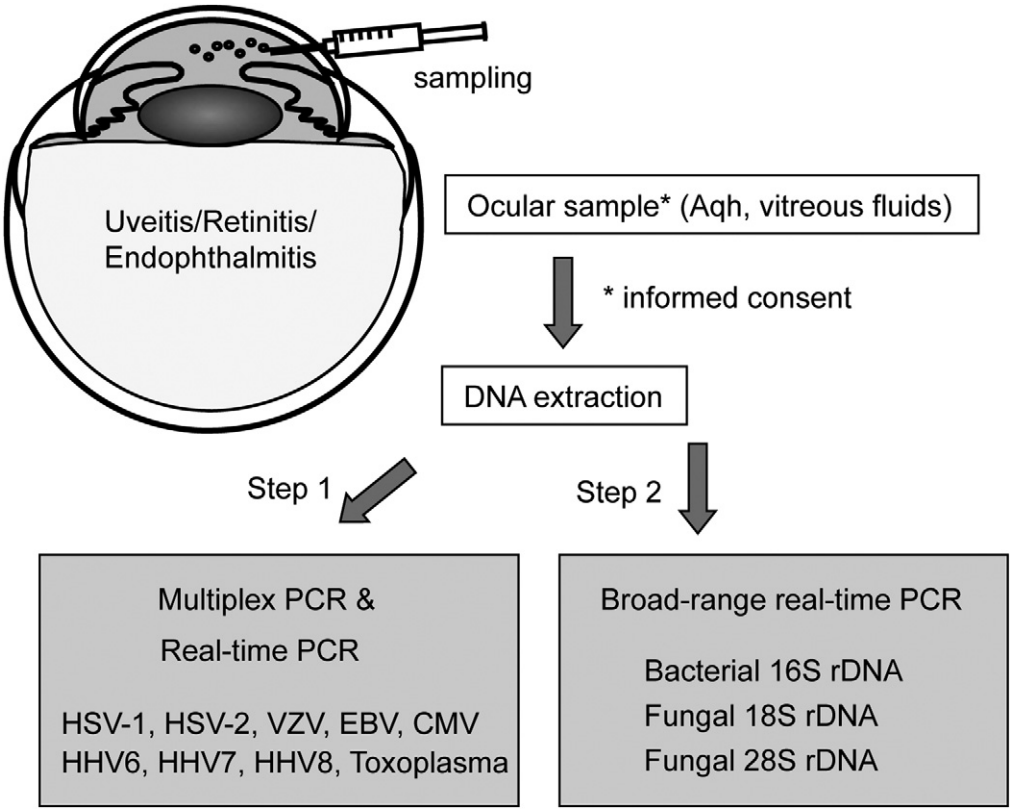

Figure 1. Diagram showing the use of a comprehensive polymerase chain reaction (PCR) system for the analysis of various infectious genomic DNA in the ocular fluids of patients with uveitis and endophthalmitis. To detect various infectious agents, we used independent PCR methods, with step 1 using multiplex PCR and real-time PCR and step 2 using broad-range real-time PCR. After DNA extraction from each of the samples, multiplex PCR was performed first to screen for human herpes virus type 1 (HHV1) to human herpes virus type 8 (HHV8) and for toxoplasmosis via the use of 3 LightCycler capillaries (Roche, Basel, Switzerland). If positive results was observed, real-time PCR was performed subsequently to measure the DNA load. Step 2 used broad-range real-time PCR for detection of bacterial 16S, fungal 18S, or fungal 28S ribosomal DNA (rDNA). To be able to detect the various bacterial and fungal DNAs, primers and probes for the unvariable regions in the sequences were used. Aqh = aqueous humor; CMV = cytomegalovirus; EBV = Epstein-Barr virus; HHV6 = human herpes virus type 6; HHV7 = human herpes virus type 7; HSV-1 = herpes simplex virus type 1; HSV-2 = herpes simplex virus type 2; VZV = varicella-zoster virus.

tious endophthalmitis, we detected 33 patients with bacterial 16S rDNA (33 of 500 cases; 6.6% positive; Table 1). In 11 patients, fungal 18S/28S rDNA was detected in the ocular fluid samples (2.2% positive). Overall, broad-range real-time PCR analysis identified 44 PCR-positive endophthalmitis patients (8.8% positive; Table 1). Analysis of the control samples from patients without intraocular inflammation (n = 100) showed that all had negative results.

Subsequently, we analyzed the results for each of the infectious antigens, including HHV-1 through HHV-8, toxoplasma, bacteria, and fungi. Table 2 shows a summary of the results. Herpes simplex virus type 1 was detected in 1 case of keratouveitis, in 16 cases of anterior uveitis, and in 1 case of ARN, whereas HSV-2 was detected in 4 cases of ARN. Varicella-zoster virus was detected in two cases of keratouveitis, 26 cases of anterior uveitis, 24 cases of ARN, and in two cases of progressive outer retinal necrosis. Epstein-Barr virus was detected in 5 cases of idiopathic uveitis. In addition, EBV also was detected in patients with various ocular inflammatory disorders and intraocular lymphoma (Table 2).

Cytomegalovirus was detected in many cases of corneal endotheliitis (11 of 12 patients), iridocyclitis (anterior uveitis; 32 of 76 patients), and CMV-associated necrotic retinitis (23 of 23 patients). Human herpes virus type 6 was detected in 2 cases of bacterial endophthalmitis. Both of these cases exhibited typical bacterial infections of the eye. Human herpes virus type 7 and HHV-8 DNA were not detected in any of the patients in this study (Table 2). Toxoplasma DNA was detected in active uveitis with ocular toxoplasmosis (n = 6), but not in any of the other ocular inflammatory cases.

Broad-range real-time PCR examinations found bacterial 16S rDNA in 26 cases of bacterial endophthalmitis, in 3 cases of idiopathic uveitis, and in 4 cases of other types of infections. Fungal 18S/28S rDNA was detected in 9 cases of fungal endophthalmitis, in 1 case of idiopathic uveitis, and in 1 case with another infection (Table 2).

We also analyzed whether this PCR examination included any false-negative or false-positive results. A false-negative result indicated that although the PCR results were negative, the patient ultimately was diagnosed with an ocular infection by other examinations or clinical findings or by their responses to treatment. Overall, we determined there were 21 false-negative results in this analysis (Table 3). Among these, the PCR results were negative in 12 cases even though the patients were suspected clinically of having bacterial endophthalmitis. However, false-positive results indicated there were positive PCR results, even though patients finally were diagnosed as having a clinically noninfectious disorder. Overall, there were 3 false-positive results for the PCR anal-

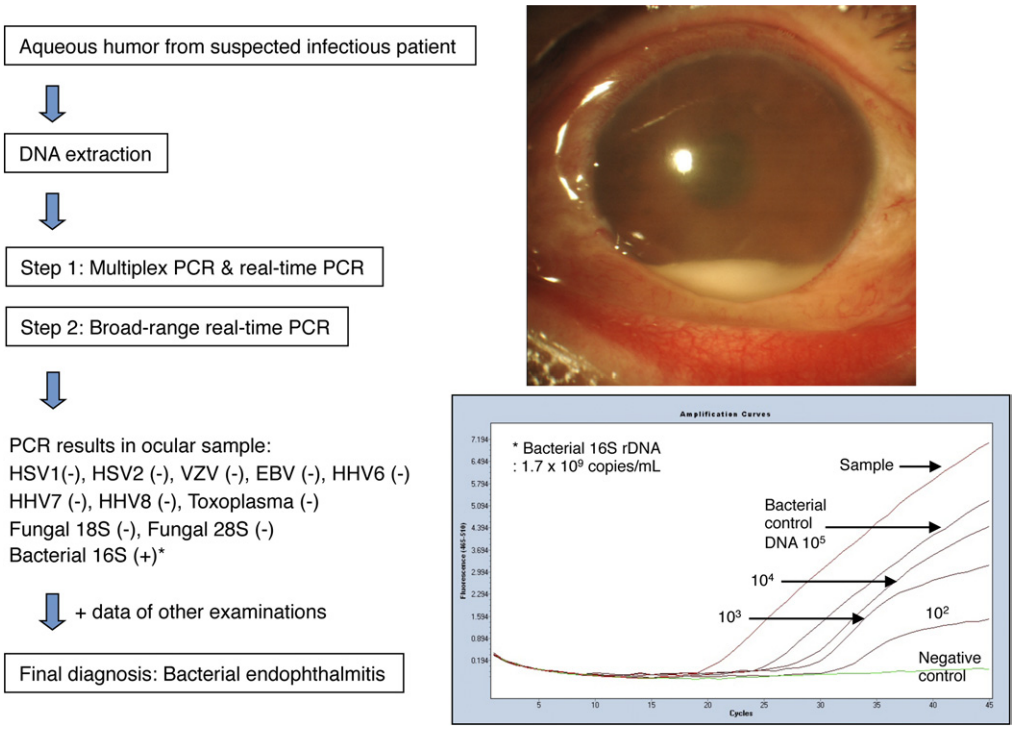

Figure 2. Polymerase chain reaction (PCR) results for a sample from a representative case. An aqueous humor sample was obtained from an endophthalmitis patient (severe anterior chamber cells with hypopyon in the slit photograph on the right). After DNA extraction from the sample, multiplex PCR was performed to screen for human herpes virus types 1 through 8 (HHV1–8) and *Toxoplasmosis gondii* using LightCycler capillaries. We simultaneously performed broad-range real-time PCR for the detection of bacterial 16S or fungal 18S/28S ribosomal DNA (rDNA) and found there was a high copy number of bacterial 16S rDNA in the sample. The sample demonstrated negative results for the human herpes viruses (HSV), such as HSV1, HSV2, varicella-zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), HHV6, HHV7, and HHV8 for *T. gondii*. Broad-range real-time PCR indicated the sample showed negative results for fungal 18S and 28S rDNA. The patient finally was diagnosed as having infectious endophthalmitis related to bacterial infections.

ysis, with each case confirmed to contain bacterial 16S rDNA (Table 4).

We also analyzed our comprehensive PCR examinations for the diagnosis of ocular infection in terms of the diagnostic parameters for sensitivity, specificity, positive predictive value, and negative predictive value. The calculated percentages for sensitivity, specificity, positive predictive value, and negative predictive value were 91.3%, 98.8%, 98.6%, and 92.4%, respectively.

Discussion

In the field of ophthalmology, PCR has proven to be very useful for patient diagnosis because the analysis can be carried out using only a very small amount of sample, such as aqueous humor. When comprehensive PCR is used, the results make it possible to include or exclude infections as the potential cause of an ocular disorder. When using our PCR system, a diagnosis can be made quickly, and in fact is much less time consuming compared with other methods. For example, multiplex PCR requires only 90 minutes, real-time PCR takes 60 minutes, and DNA extraction can be performed in only 40 minutes. Additionally, the diagnostic parameters (sensitivity, specificity, positive predictive value, and negative predictive value) of these comprehensive PCR examinations are very high. Even so, it should be noted that with regard to the sensitivity and specificity, there is no agreed upon gold standard, which could lead to difficulties when making a diagnosis. However, from a clinical aspect, use of these comprehensive PCR examinations to analyze ocular samples makes it possible to diagnose rapidly patients with unknown intraocular infectious disorders such as uveitis and endophthalmitis.

With multiplex PCR, it is possible to screen rapidly for the genome of more than 10 types of infectious antigens.[2–4] Additionally, when the PCR screening results are positive, this real-time PCR then can be used to measure the DNA load. Thus, use of this real-time PCR makes it possible to obtain quantitative information for antigen DNA in samples. Recently, broad-range real-time PCR for bacteria or fungi has become available.[1,5,6] With this method, we were able to measure the amplification of the target rDNA genes. Target antigens for this method include the bacteria 16S,[1] fungal 28S,[6] and *Candida* or *Aspergillus* 18S rRNA genes.[5] Detection of bacterial or fungal DNA is possible because primers and probes can be designed specifically for the unvariable regions (bacterial or fungal common regions) within the sequences. By using the primers and probes of these genes, broad-range PCR can be used to detect the presence of bacteria or fungi in the samples.

Table 1. Positivity of the Comprehensive Polymerase Chain Reaction System in the Ocular Fluids of 500 Patients with Uveitis and Endophthalmitis

Infectious Antigens	Multiplex PCR and Real-Time PCR	Broad-Range Real-Time PCR
HSV-1 (HHV-1)	18/500 (3.6%)	—
HSV-2 (HHV-2)	4/500 (0.8%)	—
VZV (HHV-3)	55/500 (11.0%)	—
EBV (HHV-4)	17/500 (3.4%)	—
CMV (HHV-5)	68/500 (13.6%)	—
HHV-6	2/500 (0.4%)	—
HHV-7	0/500 (0%)	—
HHV-8	0/500 (0%)	—
Toxoplasmosis	6/500 (1.2%)	—
Bacteria 16S	—	33/500 (6.6%)
Fungal 18S/28S	—	11/500 (2.2%)
Total	170/500 (34.0%)	44/500 (8.8%)

CMV = cytomegalovirus; EBV = Epstein-Barr virus; HHV-1 through HHV-8 = human herpes virus types 1 through 8; HSV-1 and HSV-2 = herpes simplex virus types 1 and 2; VZV = varicella-zoster virus.
Multiplex polymerase chain reaction (PCR) and real-time PCR were performed to screen for or detect HHV genomic DNA and toxoplasmosis. Broad-range real-time PCR was performed to screen for and detect bacterial 16S and fungal 18S/28S ribosomal DNA.

Based on PCR results, clinical findings, and other examinations, we were able to diagnose infectious diseases successfully. In the present study, with the exception of HHV-7 and HHV-8, almost all of the infectious agents were detected in collected ocular samples. Herpes simplex virus type 1 was detected in aqueous humor of patients with keratouveitis, anterior uveitis, and ARN. However, the symptoms in 4 ARN patients were shown to be related to an HSV-2 infection. In addition to detecting VZV DNA in many ocular samples, our results also indicated a high copy number of VZV DNA in the samples. We previously reported finding an association between the VZV viral load in the aqueous humor and the clinical manifestations of VZV anterior uveitis.[7] Iris atrophy was found to be much more severe in the high-viral load group compared with the low-viral load group. Overall, our results demonstrated that there was a significant correlation between the VZV viral load in the aqueous humor and damage to the iris, such as iris atrophy and pupil distortion, in patients with VZV-related anterior uveitis.

Multiplex and real-time PCR analysis of samples from various infectious patients detected EBV DNA in 17 of 500 cases (3.4%). In a previous study,[8] we found only 3 of 17 samples from uveitis patients to have significantly high EBV DNA copy numbers when using real-time PCR. Because the EBV viral load was not very high in these samples, this suggested that viral replication of EBV does not occur in the eye. It has been assumed that the EBV infection acts as a secondary factor in the pathogenesis of ocular inflammation.[9] Epstein-Barr virus has been shown to be able to infect B-lymphocytes and epithelial cells, including ocular pigment epithelial cells.[10] Therefore, it may be the intraocular infiltrating B-cells and the epithelial cells that are releasing the EBV DNA within the eye during inflammatory conditions. This is supported by our finding that EBV DNA was detected in ocular samples from patients with severe intraocular inflammatory disease such as ARN and bacterial or fungal endophthalmitis (see Table 2).

Cytomegalovirus DNA was detected in patients with corneal endotheliitis, anterior uveitis without corneal endothelium edema, and CMV retinitis. Corneal endotheliitis or anterior uveitis was not seen in immunocompromised patients. However, CMV retinitis was seen in immunocompromised patients, such as those with HIV infections. Cytomegalovirus-related anterior uveitis is similar to Posner-Schlossman syndrome, in which whitish, small, mutton-fat keratic precipitates, high intraocular pressure, and mild inflammation

Table 2. Comprehensive Polymerase Chain Reaction Results for Each Infectious Genome in Patients with Uveitis and Endophthalmitis

Infectious Antigens	Clinical Diagnosis	Positive PCR Results*/ Total No. of Patients (Mean Age of Onset [yrs])
HSV-1	Herpetic keratouveitis	1/4† (43)
	Herpetic anterior uveitis	16/76 (51)
	Acute retinal necrosis	1/29 (39)
	Others	0/391 (—)
HSV-2	Acute retinal necrosis	4/29 (28)
	Others	0/471 (—)
VZV	Herpetic keratouveitis	2/4 (61)
	Herpetic anterior uveitis	26/76 (66)
	Acute retinal necrosis	24/29 (51)
	PORN	2/2 (53)
	Others	1/389 (55)
EBV	Idiopathic uveitis	5/107 (60)
	Fungal endophthalmitis	3/11 (69)
	Bacterial endophthalmitis	1/38 (75)
	Acute retinal necrosis (VZV)	3/24 (49)
	Intraocular lymphoma	1/43 (72)
	Others	4/277 (48)
CMV	Corneal endotheliitis	11/12 (67)
	Herpetic anterior uveitis	32/76 (58)
	Cytomegalovirus retinitis	23/23 (57)
	Others	2/389 (52)
HHV-6	Bacterial endophthalmitis	2/38 (71)
	Others	0/462 (—)
HHV-7	—	0/500 (—)
HHV-8	—	0/500 (—)
Toxoplasmosis	Ocular toxoplasmosis	6/9 (55)
	Others	0/491 (—)
Bacteria 16S	Bacterial endophthalmitis	26/38 (64)
	Idiopathic uveitis	3/107 (54)
	Others	4/355 (67)
Fungal 18S/28S	Fungal endophthalmitis	9/11 (61)
	Fungal keratitis	1/1 (55)
	Others	1/488 (65)

CMV = cytomegalovirus; EBV = Epstein-Barr virus; HHV-6 through HHV-8 = human herpes virus types 6 through 8; HSV-1 and HSV-2 = herpes simplex virus types 1 and 2; PCR = polymerase chain reaction; PORN = progressive outer retinal necrosis; VZV = varicella-zoster virus.
*Detection of infectious DNA by multiplex PCR combined with real-time PCR or broad-range real-time PCR.
†In the 4 keratouveitis patients, the PCR system detected HSV-1 DNA in only 1 patient.

Table 3. False-Negative Results for Polymerase Chain Reaction

Clinical Diagnosis	False-Negative Results*
Corneal endotheliitis (cytomegalovirus)	1
Herpetic keratouveitis	1
Herpetic anterior uveitis	2
Ocular toxoplasmosis	3
Bacterial endophthalmitis	12
Fungal endophthalmitis	2

*False-negative results indicate negative polymerase chain reaction results, with a final diagnosis of ocular infection as determined by other examinations, clinical findings, or responses to treatment.

in the anterior chamber are observed.[11,12] In these types of cases, both the retina and fellow eye usually are intact. However, CMV-related corneal endotheliitis exhibits corneal endothelium edema but not anterior uveitis.[12–15] Recently, several investigators have reported finding cases of CMV-associated corneal endotheliitis when using this new PCR technique.[12,15,16] It additionally was reported that this inflammation could be well controlled through the use of antiviral agents.[12–16]

In both our previous and present studies, we observed a few cases with positive HHV-6 results. As reported previously, we also have encountered a patient with apparent severe unilateral panuveitis.[17] After further examination of this particular case, we finally determined the patient had ocular toxocariasis and HHV-6–associated panuveitis. In addition, we also found 2 HHV-6–positive cases with bacterial endophthalmitis in our present study, with neither of the patients found to be immunocompromised. Thus, at the present time there is no conclusive evidence that clarifies whether viral replication of HHV-6 occurs in the eye. Of all of the patients examined in the present study, there were no HHV-7– or HHV-8–positive cases.

This study examined many bacteria-positive endophthalmitis cases. Sample analysis led to the detection of bacterial 16S rDNA in 26 of 38 patients with clinically suspected bacterial endophthalmitis. With the exception of the PCR-negative cases, high bacterial DNA copy numbers were detected in all of these patients. Our broad-range real-time PCR detected bacterial 16S rDNA in samples from 3 patients with idiopathic uveitis, which were false-positive results (Table 4). However, bacteria 16S copy numbers were not very high in these patients. It has been suggested that amplification of bacteria species may occur in patients undergoing long-term steroid treatments. In fact, the 3 cases in our present study all had received subconjunctival injections, systemic steroids, or both over a long period. Other explanations for our present results could be contamination caused by technical errors during the PCR preparation or bacterial exposure that occurred when collecting the samples (e.g., contamination resulting from conjunctival ocular flora present when collecting the ocular sample). Other than these 3 cases, we did not observe any PCR false-positive results resulting from herpes virus, fungi, or parasites.

In this study, we used 2 PCR methods to detect fungal infections, one for fungal 18S and one for 28S rDNA. For the 18S, we designed pan-fungal primers and probes that were complementary to the 18S rRNA sequences present in the *Candida* and *Aspergillus* species.[5] Our PCR system detected 6 *Candida* species, along with 5 *Aspergillus* species. In another study, we used several different primers and probes to detect separately each of these fungal species.[5] Additionally, although our PCR examination was able to detect all species of *Candida* and *Aspergillus* DNA, it did not detect any other fungi DNA. Therefore, we prepared a separate assay that targeted a part of the 28S large subunit rRNA genes for others.[6,18] *Candida* ocular infection is very similar to endogenous endophthalmitis, and in the past, we have encountered some rare *Aspergillus*-positive cases, for example, retinal vasculitis, endogenous endophthalmitis, late postoperative endophthalmitis, and post-traumatic keratitis-associated endophthalmitis. Fungal DNA was detected in 9 of the 11 ocular samples obtained from fungal endophthalmitis patients (Table 2). One fungal keratitis case also had positive results for fungal 28S rDNA in the aqueous humor. These PCR-positive samples all had significantly high copy numbers of *Candida*, *Aspergillus*, or *Cryptococcus* DNA. In 2 patients who were clinically suspected of having *Candida* endophthalmitis, our PCR analysis did not detect any fungal genome in the ocular sample. However, it should be noted that this sample was aqueous humor, and if we had obtained a vitreous sample instead, we might have detected *Candida* DNA because *Candida* endophthalmitis often results from hematogenous dissemination. This finding suggests that the type of sample collected could be very important with regard to the ability to make an accurate diagnosis.

In our bacterial 16S PCR study, we found false-negative results in 12 of the 38 samples obtained from clinically suspected bacterial endophthalmitis patients (Table 3). The false-negative results were defined as being negative for PCR even though there was a clinically suspected bacterial infection, for example, culture positive, having an inflammation that was well-controlled by antibiotics, or both. Once again, it is necessary to consider how the samples were actually obtained in these cases. Bacterial 16S rDNA was not detected in a few of the endogenous bacterial endophthalmitis patients. However, because endogenous endophthalmitis results from hematogenous dissemination, it might have been possible to detect bacterial genome if we had collected vitreous samples. Although the proper DNA extraction procedure required for verifying bacterial infec-

Table 4. False-Positive Results for Polymerase Chain Reaction

Polymerase Chain Reaction for Infectious Antigens	False-Positive Results*
Bacteria 16S	3

*False-positive results indicate positive polymerase chain reaction results, with a final diagnosis of clinically noninfectious disease. These patients with bacteria 16S false-positive resuts ultimately were diagnosed with idiopathic uveitis.

tion by PCR remains controversial, we have attempted to use various approaches for the DNA extraction that will upregulate the PCR sensitivity. In general, a bactericidal enzyme pretreatment (e.g., lysozyme pretreatment) is required for bacterial cell wall destruction, and several investigators have reported previously finding lysozyme resistance in gram-negative and gram-positive bacteria species.[19,20] However, we did not pretreat any of our samples with enzyme because of the limited amount of sample that was available and the fact that our PCR examination included other infectious agents, such as viruses, fungi, and parasites. Therefore, it possible that bacterial 16S rDNA might not have been detected in a few of the endogenous bacterial endophthalmitis patients because of difficulties in collecting samples from patients with infectious agglomeration.

In conclusion, our results indicate that a comprehensive PCR system can be used to verify ocular disease diagnoses definitively. Furthermore, this PCR system also is able to exclude ocular infections as the potential cause of ocular disorders and, based on the confidence of the diagnosis, can be used to help design appropriate early treatments for ocular disease. Because it is important to be able to exclude noninfectious uveitis or endophthalmitis, the PCR-negative results can help to simplify the clinical workups in these cases. Additionally, because PCR examinations can be used to exclude infectious agents, this makes it easier to determine which cases are applicable for use of steroids. Although unfortunately this laboratory approach is not commercially available at the present time, we currently are pursing plans to create a simple examination kit that can be used for ocular infectious diseases in the near future.

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[1] Department of Ophthalmology & Visual Science, Tokyo Medical and Dental University, Tokyo, Japan.

[2] Laboratory for Retinal Regeneration, RIKEN Center for Developmental Biology, Kobe, Japan.

[3] Department of Virology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan.

[4] Center for Cell Therapy, Tokyo Medical and Dental University, Tokyo, Japan.

[5] Department of Ophthalmology, Osaka Koseinennkinn Hospital, Osaka, Japan.

[6] Department of Ophthalmology, Osaka University Graduate School of Medicine, Osaka, Japan.

[7] Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan.

[8] Department of Ophthalmology, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan.

[9] Department of Ophthalmology, Tokyo Medical University, Tokyo, Japan.

[10] Department of Ophthalmology, Yamaguchi University Graduate School of Medicine, Yamaguchi, Japan.

[11] Department of Ophthalmology, National Defense Medical College, Tokyo, Japan.

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Correspondence:
Sunao Sugita, MD, PhD, Laboratory for Retinal Regeneration, RIKEN Center for Developmental Biology, 2-2-3 Minatojima-minamimachi, Chuo-ku, Kobe 650-0047, Japan. E-mail: sunaoph@cdb.riken.jp.