The diagnosis of delayed post-operative endophthalmitis by polymerase chain reaction of bacterial DNA in vitreous samples

P. G. HYKIN, K. TOBAL, G. McINTYRE*, M. M. MATHESON†, H. M. A. TOWLER and S. L. LIGHTMAN

Departments of Clinical Science and † Pathology, Institute of Ophthalmology, Bath Street, London EC1V 9EL and
* Department of Medical Microbiology, University College London Medical School, London W1P 7PN

Summary. Delayed post-operative endophthalmitis is a complication of modern cataract extraction and posterior chamber lens implantation. *Propionibacterium acnes* has been isolated in a few such cases but the majority are culture-negative, compounding surgical and medical management decisions. A method of detecting bacterial, and specifically *P. acnes*, DNA by the polymerase chain reaction (PCR) directed at 16S rDNA is reported. Nested PCR with universal eubacterial primers complimentary to regions of 16S rDNA conserved sequences detected 50 fg of bacterial DNA spike in normal vitreous. Nested PCR with *P. acnes* primers detected 10 fg of DNA. Vitreous samples from 29 patients undergoing vitrectomy for reasons unrelated to infection and 23 samples from 19 patients with delayed post-operative endophthalmitis were analysed. Four (14%) of 29 normal individuals and 17 (74%) of 23 delayed cases gave positive results with universal eubacterial primers. None of 29 and eight of 23 samples gave positive results with *P. acnes* primers. The 14% positive rate with universal primers in non-infected cases may limit their use in delayed post-operative endophthalmitis. PCR detection of bacterial DNA with specific primers from vitreous samples may prove a useful means of diagnosing delayed post-operative endophthalmitis and facilitating management decisions when conventional bacterial culture is negative.

Introduction

Intra-ocular infections after ophthalmic surgery are fortunately very rare.¹ Acute post-operative endophthalmitis is a well characterised and severe complication of intra-ocular surgery, most frequently due to *Staphylococcus epidermidis*, *S. aureus*, *Streptococcus* spp., and gram-negative organisms.^{2–6} The clinical course is rapidly progressive and the prognosis generally poor, despite aggressive surgical and antibiotic therapy.^{7,8} The occurrence of delayed post-operative endophthalmitis has been recognised increasingly in recent years and typically follows extracapsular cataract extraction with posterior chamber lens implantation.^{9–16} It occurs 1–11 months after surgery, is characterised by repeated episodes of low grade inflammation—which in the early stages may respond to topical steroid treatment—and clinically by large keratoprecipitates, hypopyon, white plaques on the posterior capsule, a capsular abscess and anterior vitritis.^{11–13, 15–17} Although *Propionibacterium acnes*^{9–16} and *P. granulosum*,^{17, 18} *P. propionicus* (*Arachnia propionica*),¹⁷ *S. epidermidis*,^{17, 19, 20} *Actinomyces israelii*,²¹

*A. viscosus*²¹ and *Corynebacterium* sp.²² have been reported in such cases, the majority are culture-negative. Reasons for this include small numbers of causative organisms present within the eye, the possible localised nature of the infection leading to sampling error and the fastidious growth requirements of *Propionibacterium* spp. This results in a clinical dilemma in which post-operative inflammation due to phaco-anaphylactic uveitis, idiopathic post-operative inflammation or delayed post-operative endophthalmitis²³ cannot be distinguished easily. Decisions regarding the appropriate form and timing of surgical intervention and the use of systemic and topical steroids and antibiotics become difficult.^{10, 11, 16, 17} To minimise these problems, a method of detecting bacterial DNA in vitreous samples is described based on the polymerase chain reaction (PCR) amplification of bacterial 16S rDNA.²⁴ It has potential advantages in that only a very small sample is required for analysis, minute numbers of bacteria can be detected and conventional microbial culture is not required.²⁵ In this study, universal eubacterial primers²⁶ from regions of conserved sequence were employed to determine whether a vitreous sample contained bacterial DNA, and primers specific to *P. acnes*, in the context of delayed post-operative endophthalmitis, were used to

Table I. NCTC and identification strains of bacteria responsible for acute and delayed post-operative endophthalmitis

<i>P. acnes</i> strains	Delayed post-operative endophthalmitis	NCTC no.	Acute post-operative endophthalmitis	NCTC no.
NCTC 737				
Identification strains:	<i>S. epidermidis</i>	11407	<i>S. aureus</i>	8532
A160/87	<i>P. granulosum</i>	11865	<i>Str. pyogenes</i>	8198
A234/85	<i>P. propionicus</i>	11666	<i>Str. pneumoniae</i>	7465
A155/85	<i>A. israelii</i>	10236	<i>Str. sanguis</i>	7863
A5/87	<i>A. viscosus</i>	10951	<i>Serratia marcescens</i>	10211
A58/87	<i>C. xerosis</i>	7238	<i>Ps. aeruginosa</i>	10322
A195/87	<i>C. pseudodiphtheriae</i>	11136	<i>Pr. mirabilis</i>	660
A222/87			<i>Bacillus cereus</i>	2599
A245/87			<i>Haemophilus influenzae</i>	8143
A253/87			<i>Klebsiella pneumoniae</i>	9633
A123/89			<i>Enterococcus faecalis</i>	775

detect the presence of this organism in vitreous samples.

Materials and methods

Clinical samples

Vitreous samples were obtained from 29 control patients undergoing three-port pars plana vitrectomy. The indications for surgery included retinal detachment and vitreous haemorrhage; in no case was infective endophthalmitis suspected. Twenty-three vitreous samples were obtained from 19 patients with delayed post-operative endophthalmitis. Samples were obtained under sterile operating conditions either by passing a needle attached to a 2-ml syringe through the pars plana of the eye, or by passing a needle through a pre-existing pars plana vitrectomy port at the commencement of the vitrectomy procedure, slowly withdrawing 250 µl of vitreous, placing 100 µl in a pre-sterilised microfuge tube for PCR, and using 150 µl for conventional microbiological analysis.

Gram's stain and culture

Gram's stain was performed on representative smears from all infected cases. Clinical specimens, infected and non-infected, were cultured at 37°C on blood agar, incubated aerobically and anaerobically, chocolate agar incubated in air with CO₂ 5% and in Robertson's cooked meat broth. Liquid phase cultures were subcultured at 48 h on to blood agar for aerobic and anaerobic incubation; anaerobic cultures were maintained for 7 days.

DNA extraction

Vitreous (50 µl) was added to 150 µl of suspension buffer (10 mM Tris-HCl, pH 8.3, 2.5 mM MgCl₂, 50 mM KCl), phenol 200 µl, 20 µl of SDS 20% and 100 µl of 100-µm diameter glass beads and agitated for 2 min in a Mini-Beadbeater[™] (Biospec products).²³ The sample

was centrifuged for 1 min at 15 600 *g* and the aqueous phase was removed, from which DNA was precipitated with ethanol (2 volumes) and 3M sodium acetate, pH 5.2 (0.1 volumes), washed with ethanol 70%, dried in a vacuum dessicator and reconstituted in 10 µl of TE buffer; 5 µl were added to the PCR reaction.

DNA was extracted from NCTC (National Collection of Type Cultures, 61 Colindale Avenue, London) and identification strain organisms (table I) by suspending the bacteria in 400 µl of TEST buffer (10 mM Tris-HCl, pH 8.0, 5 mM EDTA, pH 8.0, 1 mM NaCl, Triton X100 0.5%); lysozyme was added to a final concentration of 2 mg/ml and the suspension was incubated at 37°C for 2 h, followed by the addition of proteinase K (20 mg/ml) 8 µl and 40 µl of N-lauryl sarcosine (sarkosyl) and incubation at 37°C for 1 h. An equal volume of phenol: chloroform: isoamyl alcohol was added and centrifuged at 15 600 *g* for 2 min; the aqueous phase was removed²⁷ and nucleic acids were precipitated with ethanol (2 volumes) and 3 M sodium acetate, pH 5.2 (0.1 volumes), and redissolved in 10 µl TE buffer. The DNA concentration was adjusted to 5 ng/µl; 2 µl of sample (10 ng) were added to the PCR reaction.

Oligonucleotide primer design

Universal eubacterial oligonucleotide primers. Highly conserved regions of 16S rDNA were identified^{24, 28, 29} and primers (U1 and rU4) and (U2 and rU3) (table II) were synthesised (394 Synthesiser) and purified by HPLC (Oswells) to generate a 766-bp (round 1) and a 470-bp (round 2) product. The primers had broad specificity for gram-positive and -negative bacteria known to cause infective endophthalmitis (table II).

***P. acnes* oligonucleotide primers.** Multiple alignment³⁰ of 16S rDNA sequences of *P. acnes*, *P. propionicus*, *A. israeli*, *A. viscosus* and *C. xerosis* identified areas of low sequence homology, which included previously characterised variable regions V1, V2 and V3.²⁷ *S. epidermidis* 16S rDNA sequence information was unavailable. Primer Pa1 was synthe-

Table II. Universal eubacterial and *P. acnes*-specific oligodeoxynucleotide primers

Primer	Sequence (5'-3')	Position in <i>E. coli</i> 16S rDNA
U1	TTGGAGAGTTTGATCCTGGCTC	4-25
U2	GGCGTGTCTTAACACATGCAAGTCG	41-64
rU3	GCGGCTGGCACGTAGTTAG	506-528
rU4	GGACTACCAGGTATCTAA	789-806
Pa1	AAGGCCCTGCTTTGTGG	71-88
rPa2	TCCATCCGCAACCGCCGAA	199-216
rPa3	ACTCACGCTTCGTACAG	442-459

sised from *P. acnes* region V1, rPa2 from region V2 and rPa3 from region V3 (table II). Pa1 and rPa3 gave a 387-bp product, and Pa1 and rPa2 a 160-bp product. These *P. acnes* primers did not amplify DNA from other causative organisms of delayed post-operative and acute endophthalmitis (table III) except *A. israelii*, an extremely rare cause of delayed post-operative endophthalmitis. The 160-bp product of these two organisms has a base-pair difference (*Escherichia coli* position 93), contained in the recognition site of restriction endonuclease *XhoI*, which was employed to differentiate them.

Prevention of contamination

Sample agitation and PCR preparation were performed in one laboratory, clinical sample addition in a second laboratory and PCR was performed and products analysed in a third laboratory. PCR preparation was performed in a laminar flow cabinet with single-use aliquots of each reagent, dedicated pipettes and plugged tips. Microfuge tubes and mineral oil aliquots were double autoclaved before use. Each universal eubacterial sample was treated with 1 unit of DNAase 1 (Boehringer) at 37°C followed by 10 min at 85°C before addition of primer, to remove inherent bacterial contamination of *Taq* DNA polymerase.³¹ Before the addition of template, samples were exposed to short wave UV light for 10 min.

PCR optimisation

Universal eubacterial oligonucleotide primers. A 50- μ l reaction was optimised with 1 unit of *Taq* DNA polymerase (Stratagene), 200 μ M of each deoxynucleotide triphosphate, 5 μ l of $\times 10$ buffer (500 mM KCl, 100 mM Tris-Cl, 15 mM MgCl₂, gelatin 0.1%, pH 8.3), 0.36 μ M of each primer, 5 μ l of template (10 ng of DNA, *c.* 10⁶ genome copies) and water was added to 50 μ l. The reaction was overlaid with 50 μ l of mineral oil and performed in a Quattro TC 140 thermocycler. The reaction was heated for the first cycle to 94°C for 5 min, annealing was performed at 60°C (round 1), and 58°C (round 2), for 1 min and extension at 72°C for 2 min. Subsequent cycles, 34 (round 1) and 24 (round 2), consisted of 94°C for 30 s, 60°C (round 1),

58°C (round 2), for 1 min and 72°C for 2 min, with a final extension at 72°C for 8 min.

***P. acnes* oligonucleotide primers.** Pa1, rPa2 and rPa3 primer concentration was 1 μ M, 2 units of *Taq* DNA polymerase were added and the temperature profile was 94°C for 5 min, 62°C (rounds 1 and 2), for 1 min and 72°C for 2 min. There were 34 (round 1) and 24 (round 2) subsequent cycles consisting of 94°C for 30 s, 62°C (rounds 1 and 2) for 1 min and 72°C for 2 min, with a final extension at 72°C for 8 min.

PCR specificity

Universal eubacterial oligonucleotide primers were employed to amplify 16S rDNA from all principal organisms known to cause acute and delayed post-operative endophthalmitis (table I). *P. acnes* oligonucleotide primers were employed to amplify *P. acnes* DNA from NCTC strain 737, from 11 *P. acnes* identification strains and from organisms known to cause acute and delayed post-operative endophthalmitis. PCR was performed with all primer combinations *versus* human nuclear DNA to ensure no cross reactivity.

PCR sensitivity

Ten-fold serial dilutions of DNA extracted from *S. epidermidis* in water and non-infected vitreous and of DNA extracted from serial dilutions of organisms suspended in phosphate-buffered saline were amplified with universal primers U1 and rU4, and U2 and rU3. One μ l of the first round was used in the second round of amplification. Ten-fold dilutions of *P. acnes* DNA in water and in normal vitreous were amplified with Pa1 and rPa3, and Pa1 and rPa2. The sensitivity of each PCR procedure was assessed after the first and second rounds of amplification.

PCR analysis of clinical specimens

Five μ l of DNA from each clinical sample were added directly to the PCR reaction. Each clinical specimen was amplified in the presence of negative controls, for the sample agitation and the PCR steps, and positive controls (5 pg and 50 fg of *P. acnes* DNA). A negative result was confirmed by spiking the clinical specimen with *P. acnes* DNA, and repeating the PCR to exclude the possibility of a false-negative result due to the presence of PCR inhibitors in the vitreous sample. The PCR products were separated by electrophoresis in agarose 1.5% gel containing ethidium bromide 0.5 μ g/ml and visualised with a transilluminator at 302 nm.

XhoI restriction endonuclease digest

The 160-bp DNA PCR products of *P. acnes* and *A. israelii* were distinguished by incubation at 37°C in the presence of 10 units of *XhoI* (Pharmacia) and endo-

Table III. Primers Pa1, rPa2, rPa3 with complimentary, 16S rDNA sequences from species known to cause delayed and acute post-operative endophthalmitis

Bacterial species	5' Pa1 3' AAGGCCCTGCTTTTGTGG	5' rPa2 3' TCCATCCCCAACCGCCGAA	5' rPa3 3' ACTCACGCTTCGTCACAG
Delayed endophthalmitis			
<i>A. israelii</i>	NA	XCXATCCCCAACCGCCGAA	ACTCACGCTTCGTCACAG
<i>P. propionicus</i>	AAGGCC-----T-TCG	TCCATCCCACACCGCAAAA	ACTTGCCTTCGTCCTTA
<i>A. viscosus</i>	GGGAGCCAGCTXXXXXX	CCXATCCCCACCGGAXCT	ACCXA*C*GCCT*CTTCACAT*G
<i>C. xerosis</i>	AAGGCCAGCTTGC-TGG	TCCATCCCACACCG-AAAA	AGAAAACTTCGTCGATGG
Acute endophthalmitis			
<i>Str. pneumoniae</i>	NA	TCCATCTGGTAGTGATGCA	A*CTCACACTC*TTCTTCTCTT
<i>Str. sanguis</i>	AGGAGCTTGC--CT-TGG	TCCATCTGGCAGTGATGCX	A*CTCACACCC*TTCTTCTCTT
<i>Str. pyogenes</i>	NA	TCCATCTCATAGTGXAGCA	A*CCCACCATCATTTCTTCTTA
<i>Ps. aeruginosa</i>	GGGAGCTTGC--CT-TGG	CTCATCTGATAGCG-TGAG	A*TTACTGCCCTTCCTCCCAA
<i>Citrobacter freundii</i>	AGGAGCTTGCTCCT-TGG	CACATXXGATGGCA-AGAG	A*CTCAACXCCTTCCTCCTCG
<i>H. influenzae</i>	GAAAGCTTGCTTTC-TTG	CTCATCTATGGCA-TGCG	A*ACATCAACCTTCCTCAATA
<i>Ser. marcescens</i>	GGGAGCTTGCTCCC-TGG	CACATCTGATGGCA-AGAG	A*CTCACCACCTTCCTCCTCG

NA, not available; X, indeterminate base; -, base deletion; *, base insertion (number and type not shown).

nuclease digest buffer (10 mM Tris acetate, pH 7.5, 10 mM Mg acetate, 50 mM K acetate). The products were analysed by electrophoresis in polyacrylamide 6% gels containing ethidium bromide 0.5 µg/ml.

Results

PCR specificity

Universal primers, U1 and rU4, and U2 and rU3 amplified successfully DNA from all recognised pathogens in acute and delayed post-operative endophthalmitis to give *c.* 766-bp and *c.* 470-bp fragments. Human nuclear DNA was not amplified. *P. acnes* primers Pa1 and rPa3, and Pa1 and rPa2 amplified DNA from all 11 strains of *P. acnes* and *A.*

israelii. DNA from other organisms known to cause delayed and acute post-operative endophthalmitis was not amplified (figs. 1 and 2). Weak amplification of *A. viscosus* DNA occurred with Pa1 and rPa2 at an annealing temperature of 58°C but not at 62°C.

PCR sensitivity

After prior DNAase 1 treatment, DNA amplification with U1 and rU4 for 35 cycles permitted detection of 1 pg of *S. epidermidis* DNA in TE buffer and in normal vitreous. With nested PCR (U2 and rU3) for a further 25 cycles, 50 fg of *S. epidermidis* DNA in TE buffer and in normal vitreous was detected. Without prior DNAase 1 treatment, DNA amplification with Pa1 and rPa3 for 35 cycles per-

Fig. 1. Nested PCR results with Pa1, rPa3. Lane 1, negative control; 2 and 13, 100-bp mol. wt marker; 3, *S. epidermidis*; 4, *P. acnes*; 5, *P. granulosum*; 6, *P. propionicus*; 7, *S. aureus*; 8, *C. xerosis*; 9, *C. pseudodiphtheriae*; 10, *A. viscosus*; 11, *P. acnes*; 12, *A. israelii*.

Fig. 2. Nested PCR results with Pa1, rPa2. Lane 1, negative control; 2 and 13, 100-bp mol. wt marker; 3, *S. epidermidis*; 4, *P. acnes*; 5, *P. granulosum*; 6, *P. propionicus*; 7, *S. aureus*; 8, *C. xerosis*; 9, *C. pseudodiphtheriae*; 10, *A. viscosus*; 11, *P. acnes*; 12, *A. israelii*.

Table IV. Gram's stain, vitreous culture and PCR results from 19 patients presenting with a clinical diagnosis of delayed pseudophakic endophthalmitis

Patient no.	Eye R/L	Procedure at diagnosis	Gram's stain	Vitreous culture	PCR results			
					EUP	IUP	EPAP	IPAP
1	L	Capsule biopsy	Pleomorphic positive rods	<i>P. acnes</i>	+	+	+	+
1	L	Capsule biopsy	Pleomorphic positive rods	<i>P. acnes</i>	+	+	+	+
2	L	Vitrectomy	Negative	<i>Citrobacter freundii</i>	+	+	-	-
3	R	Vitrectomy	Negative	Negative	+	+	-	-
4	R	Vitrectomy	Negative	Negative	+	+	-	-
5	L	Vitrectomy	Negative	Negative	-	-	-	-
6	L	Vitrectomy	Negative	Negative	+	+	-	-
7	L	Vitrectomy	Negative	Negative	-	-	-	-
8	R	Vitrectomy	Negative	Negative	-	-	-	-
9	R	Vitrectomy	Negative	<i>Streptococcus</i> Group D	+	+	-	-
10	R	Vitreous biopsy	Negative	Negative	-	-	-	-
11	R	Vitrectomy	Negative	<i>S. epidermidis</i>	+	+	-	-
12	L	Vitrectomy	Negative	Negative	-	-	-	-
13	L	Vitreous biopsy	Positive cocci	<i>S. epidermidis</i> <i>S. aureus</i>	+	+	-	-
14	R	Vitreous biopsy	Positive cocci	<i>S. epidermidis</i>	+	+	+	+
15	L	Vitreous biopsy	Negative	Negative	-	+	-	+
15	L	Vitrectomy	Negative	Negative	-	+	-	+
16	R	Vitreous biopsy	Pleomorphic positive rods	<i>P. acnes</i>	+	+	-	+
16	R	Vitrectomy	Negative	<i>P. acnes</i>	+	+	+	+
17	L	Vitreous biopsy	Negative	Negative	-	-	-	-
18	L	Vitrectomy	Negative	Negative	-	+	-	-
18	L	Capsule biopsy	Negative	Negative	+	+	-	-
19	L	Vitrectomy	Pleomorphic positive rods	Negative	-	+	-	+

EUP, external universal primers; IUP, internal universal primers; EPAP, external *P. acnes* primers; IPAP, internal *P. acnes* primers; R, right eye; L, left eye.

mitted detection of 1 pg of *P. acnes* DNA in TE buffer and in normal vitreous. With nested PCR (Pa1 and rPa2) for a further 25 cycles, 10 fg of *P. acnes* DNA in TE buffer and in normal vitreous was detected.

*Xho*I restriction endonuclease digest

*Xho*I restriction endonuclease digest of the 160-bp nested PCR production of *P. acnes* produced a 135-bp

and a 25-bp product. The *A. israelii* nested PCR product was not cleaved by the enzyme.

Clinical specimens

Of 29 control vitreous samples, four gave positive results after nested PCR with universal primers, but none was positive after nested PCR with *P. acnes* primers. In all cases, conventional microbial culture of vitreous specimens was negative. Of 23 vitreous samples from 19 patients with delayed post-operative endophthalmitis, 17 gave positive results with nested universal primers, and eight with nested *P. acnes* primers (table IV). No sample positive by conventional microbial culture gave negative results by PCR either with universal or *P. acnes* primers. All four samples from which *P. acnes* was isolated clinically gave positive results by PCR, as did a case in which *P. acnes* was suspected on clinical grounds and seen on Gram's stain but which was culture negative. In addition to this, three further cases in which the clinical diagnosis of *P. acnes* delayed post-operative endophthalmitis was suspected, but culture for the organism was negative, were PCR positive. *S. epidermidis* was cultured from one of these cases. Nine vitreous specimens that gave positive results with the universal primers gave negative results by PCR with *P. acnes* primers; of these, four were culture positive (table IV; *S. epidermidis* 2, *Citrobacter freundii* 1, *Streptococcus* group D 1).

Discussion

This study was designed to answer two specific clinical questions. First, whether PCR could be employed to identify low grade infections in vitreous samples with universal primers or primers specific for *P. acnes*. Second, whether the detection by PCR of cases of *P. acnes* endophthalmitis which are negative by culture would help to indicate the true incidence of low grade bacterial endophthalmitis. At present, cases are seen in which persistent recurring low grade inflammation is present after surgery that could be of bacterial origin, but this is not confirmed by conventional microbiological techniques. Scanning electronmicroscopy of intra-ocular lenses explanted from such cases has demonstrated bacteria adherent to the implant optic and haptics of such cases.²³ Furthermore, it is possible that bacteria as yet unrecognised as intra-ocular pathogens are present in some of these cases. DNA sequencing of the universal nested PCR product may allow identification of the causative organism in a number of such cases.

Universal primers were chosen from 16S rDNA because of the large amount of available sequence information³²⁻³⁴ and because highly conserved regions of the gene allow for synthesis of primers that amplify a wide spectrum of bacteria.²⁹ Successful amplification of DNA from all bacteria implicated in delayed and acute post-operative endophthalmitis was obtained

with these primers, confirming the high homology between the universal primers and all bacterial 16S rDNA template sequences.

16S rDNA was chosen as the target gene for *P. acnes* primers because it is the only published sequence for this organism. Over 97% sequence homology between the 16S rDNA of *P. acnes* and *A. israelii*, a rare cause of delayed post-operative endophthalmitis, meant that it was not possible to construct primers completely specific to *P. acnes*, even from areas of low sequence homology within the gene (variable regions V1-V6). However, the base-pair difference between *P. acnes* and *A. israelii* in the nested (2nd round) product (*E. coli* position 93) lies in the recognition site for restriction endonuclease *Xho*I, permitting differentiation of the two bacterial sequences. Of the other organisms causing delayed post-operative endophthalmitis, significant differences between the *P. acnes* primers and 16S rDNA templates were present in all cases except between rPA2 and the V2 region of *A. viscosus*, with only two mismatches and two deletions. Despite the apparent low number of primer-template mismatches in this instance, no non-specific amplification occurred in the nested PCR with an annealing temperature of 62°C.

As neither the universal nor *P. acnes* primers amplified human nuclear DNA, and human mitochondrial DNA has no sequence homology to primer G1, a false positive result from non-specific amplification of human DNA was not expected.

The sensitivity of the universal primers was such that 50 fg—approximately 10 genome copies—of *S. epidermidis* DNA could be detected in vitreous. This approximates to the sensitivity achieved in other studies and supports the belief that PCR is not inhibited by vitreous humour in a template sample.³⁵ The sensitivity of the *P. acnes* nested PCR was five-fold more than PCR with the universal primers.

Preparation of clinical vitreous samples for PCR by boiling,³⁶ treatment with 0.1 M NaOH³⁷ and proteinase K extraction followed by phenol: chloroform extraction and ethanol precipitation was unsuccessful. This was thought to be due to the recalcitrant nature of organisms such as *Propionibacterium* spp. and *Actinomyces* spp.³⁸ with these methods of preparation. The Mini-Beadbeater[™] was employed to lyse bacterial cells suspended in phosphate-buffered saline and was followed by successful PCR amplification of the lysed sample. The Mini-Beadbeater has been used previously for quick and effective DNA extraction for taxonomic purposes²⁸ but has not been described previously in a clinical application.

In none of 29 control cases that underwent vitrectomy had previously surgery been performed. There is no evidence that normal vitreous contains bacterial DNA—this implies that the four positive results with universal primers represent pick-up of bacteria from the ocular surface at the time of vitreous sampling. This positivity rate or 14% in clinically non-infected cases is higher than in studies of PCR in meningococcal

meningitis³⁹ and acute retinal necrosis³⁵ and probably reflects an increased risk of positive results with universal primers. Spiking of all negative samples with *P. acnes* DNA confirmed that positive results were obtainable in all cases, despite the fact that vitreous samples contained blood in four cases. The fact that a positive result was possible in all 29 cases after spiking with *P. acnes* DNA suggests that bacteria other than *P. acnes* in the conjunctival sac^{40,41} may have been responsible for the four positive results with the universal primers in the non-infected group, despite the fact that this organism is frequently isolated from lid cultures.⁴²

The patients in the delayed post-operative endophthalmitis group were selected on the basis of low grade inflammation occurring at least 1 month after surgery. Not every case had all the typical clinical characteristics of *P. acnes* endophthalmitis, but it was felt that this was the most appropriate group in which to look for the organism. Positive PCR results with universal primers corresponded to cases that were positive by Gram's stain or bacterial culture, or both; no culture positive case gave negative PCR results with universal primers. Within this group, cases culture positive for *P. acnes* gave positive PCR results. One case had a Gram's stain consistent with *P. acnes*, was culture negative but PCR positive, and three further culture negative cases were PCR positive. In one of these cases, *S. epidermidis* was cultured—suggesting that *P. acnes* may have been present as part of a mixed infection. It appears that the PCR is capable of detecting *P. acnes* in certain cases of delayed post-operative endophthalmitis in which the organism is not detected by other means. Larger numbers of samples are required to test these preliminary findings further.

The localised nature of *P. acnes* endophthalmitis around the capsular bag suggests that vitreous sam-

pling may fail to detect the organism. This theory does not appear to have been supported by the data presented here, a minute number of organisms present in the vitreous biopsy being sufficient for a positive PCR result. Capsule biopsy and fluid from the capsular bag are now routinely taken in these cases to minimise sampling error.

Identification of *P. acnes* by PCR in cases which were culture negative did not alter immediate management. However, it prevented subsequent persistence with systemic steroids in one case, with earlier recourse to further surgery. In cases in which vitrectomy is performed initially and intravitreal antibiotics given, but in which conventional cultures are negative, a positive PCR result in the face of further inflammation in the capsular bag may result in earlier further surgical intervention. Mild intra-ocular inflammation managed with a vitreous tap and intravitreal antibiotics with negative cultures but a positive PCR result may make the clinician less likely to persist with topical and oral steroids and consider early surgery.

Although numbers in this study were small, PCR amplification with *P. acnes* primers followed by *Xho*I restriction endonuclease digest is a sensitive way of detecting minute quantities of *P. acnes* in vitreous and may alter clinical management of some cases. The positive results with universal primers amongst control cases require further evaluations. However, the universal primers provide a useful starting point for the detection of bacterial DNA from otherwise undiagnosed cases of endophthalmitis. Cloning and sequencing of the 16S rDNA PCR products from these cases may permit identification of the pathogen involved.

We acknowledge the expert advice received from Dr R. A. Cox, National Institute for Medical Research, Mill Hill and Mr J. K. G. Dart, Moorfields Eye Hospital, London, in the preparation of this manuscript.

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