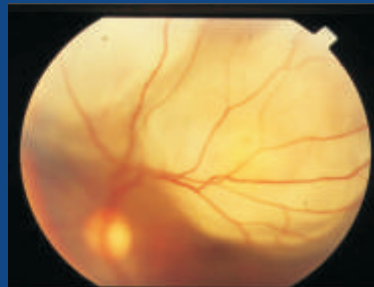
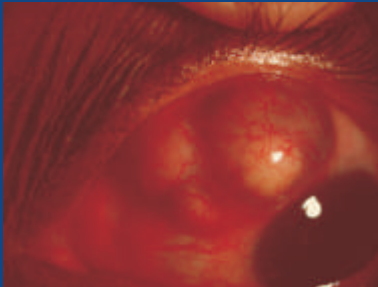
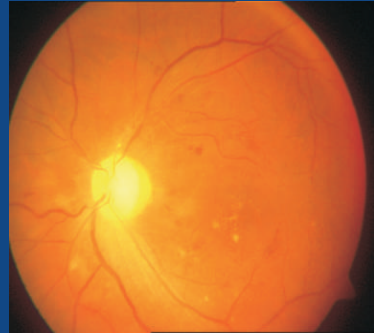
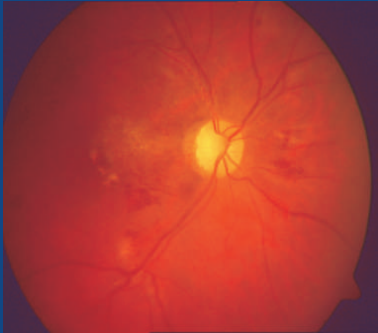




AIOS, CME SERIES (No. 22)

Polymerase Chain Reaction in Ophthalmology

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ALL INDIA OPHTHALMOLOGICAL SOCIETY

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About CME

PCR is one of the more recent tests in the diagnostic armamentarium of Ophthalmologists. Though used by many, not all understand / employ it judiciously.

This booklet, by the doyens in the field, besides telling the reader about the basics of PCR, details the current applications of PCR in Ophthalmology. The hallmark is simple Lucid language, because of which you can finish reading in one go and become wiser.

Congratulation to all the authors !!

Rajvardhan Azad
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Foreword

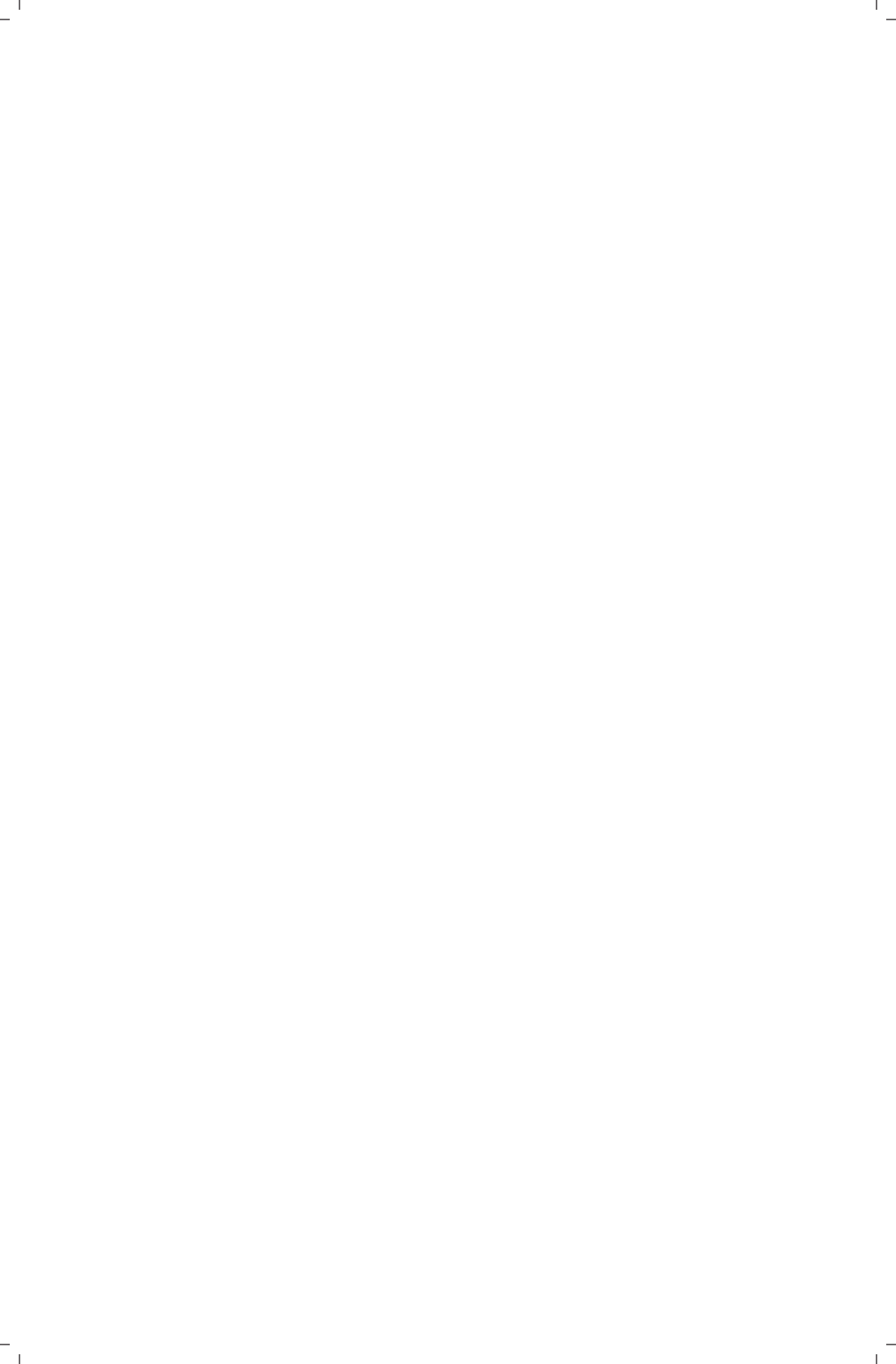
Dear colleagues,

Polymerase chain reaction (PCR) has become a vital diagnostic tool in Ophthalmology. It is now widely used in the early diagnosis of different infective diseases of the eye. PCR is used to analyse aqueous and vitreous samples for different micro-organisms in infective uveitis, viral retinitis, endophthalmitis etc. It is also helpful in diagnosing different viral strains causing conjunctivitis, keratitis and keratoconjunctivitis. It has a distinct advantage over conventional diagnostic methods as it offers rapid diagnosis at lower organism loads. Thus it is of immense benefit to ophthalmologists in management of conditions like endophthalmitis, viral retinitis etc.

In this CME series titled “PCR in Ophthalmology”, Dr Jyotirmoy Biswas has presented the basics of this diagnostic tool in a simplified and illustrated manner. I am sure it will benefit all ophthalmologists.

I would like to appreciate the efforts of Dr Biswas in compiling an excellent practical guide on Polymerase Chain Reaction in Ophthalmology.

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Polymerase Chain Reaction in Ophthalmology

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Introduction

The polymerase chain reaction (PCR) is a technique of selectively amplifying a single or few copies of a piece of DNA, thereby generating millions or more copies of a particular DNA sequence. So, broadly PCR can be described as “molecular photocopier” or more simply it can be termed as “DNA replication in a test tube”.

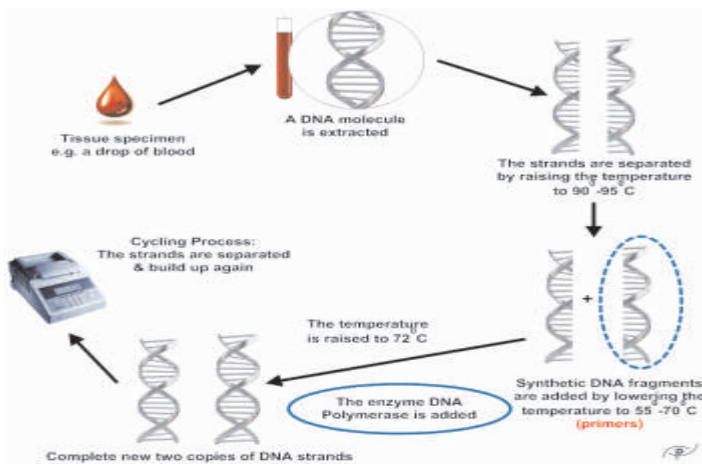
Polymerase chain reaction was invented by Kary Mullis of USA¹ in 1983 for which he was awarded the Nobel Prize in chemistry in 1993. However, the basic principle of replicating a piece of DNA using two primers had already been described by Gobind Khurana in 1971².

The PCR is superior in terms of sensitivity, specificity and rapidity of other diagnostic tests in the armamentarium. The presence of DNA or RNA of the pathogen can directly be detected without waiting for the in-vitro culture results. But the usefulness of this test is dependent on careful judgement of the ophthalmologist, correlating with the clinical diagnosis.

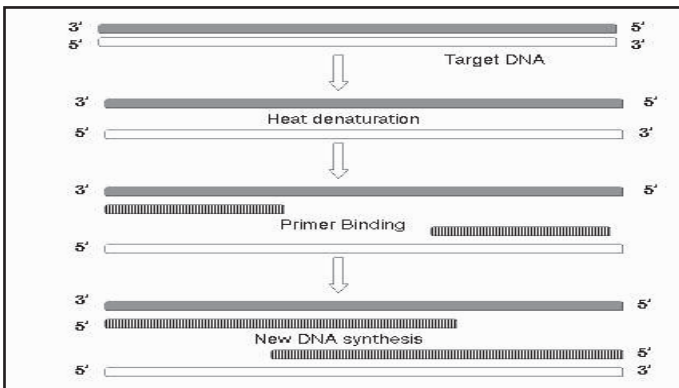
Polymerase chain reaction was initially utilized for research purposes, but over the years, it has emerged as a powerful diagnostic technique especially for detecting the presence of infectious agents which may be difficult to be grown on culture media. They may be very useful to detect the presence of slow growing known causes of ocular infections.

Kary Mullis
Inventor of PCR





PCR process at a glance



Biochemical basis of PCR



Biochemical Basis of PCR^{3, 4, 7}

Materials or reagent required for PCR:

1. Biopsy specimens or specimens containing template or target DNA-DNA sequence to be amplified
2. Primers- single stranded DNA oligonucleotides (20-50 nucleotides long)
3. DNA nucleotides, building block for new DNA
4. DNA polymerase-as it is necessary to increase the temperature to separate the strands of double stranded DNA in each cycle of amplification process, a thermostable DNA polymerase (Taq polymerase) is used which was originally isolated from a bacterium – *Thermus aquaticus*
5. Buffered salts

Instruments used for PCR: PCR machine or Thermocycler for automated thermal cycling and analysing PCR.

Process: Broadly each cycle of PCR consists of 3 stages :

- **Denaturation** = samples of interest is heated (90° - 95° C) to denature the double stranded DNA thereby producing single stranded DNA.
- **Primer Binding** = two short oligonucleotide primers (15-20 nucleotides) are used to bind to its associated complementary sequence with single stranded DNA, separated in the previous cycle. These primers are signature sequences specific for the organism of interest. The specificity of primer binding is the basis of specificity of the diagnostic test.
- **DNA Synthesis** = DNA polymerase is used to catalyse the synthesis of target sequence from the annealed primers produced in previous step.

Generally 30-40 such cycles are used for PCR assays which corresponds to $>10^{10}$ amplifications of starting DNA material.

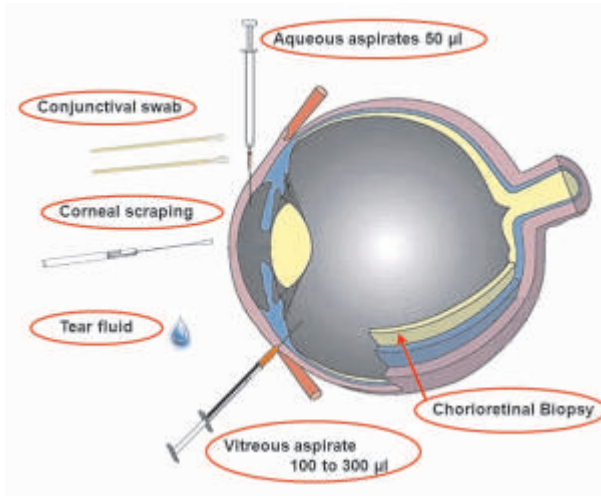
End products of PCR assay can be seen by separating DNA molecules by size using 2% agarose gel or acrylamide gel electrophoresis and then various dyes like ethidium bromide can be used to stain the resultant gel.

Time: usually 2-5 hrs

Controls used in PCR: Positive control: includes known amount of standard strains of pathogen DNA

Negative control (Reagent control): includes all reagents except for patients DNA samples which are substituted by pure distilled water.

Sample Required for PCR ^{3, 4, 7}:



Samples Required for PCR

Any tissue or body fluid can be used for PCR. In modern day ophthalmology practice, the samples for PCR are usually obtained from conjunctival swab, anterior chamber paracentesis or vitreous aspiration. Tear fluid, corneal epithelial scrapings and conjunctival swab or scraping also can be used to perform PCR. Tear fluid can be collected from eye wash by rinsing the ocular surface with 500 µl of sterile saline.²⁹ The choice of collecting sample should be guided by disease suspicion. For aqueous aspirates, 50 µl of intraocular fluid is usually sufficient for performing the procedure whereas 100 to 300 µl of vitreous aspirate is sufficient for diagnostic purpose. Specimen should be aseptically transferred to a new, sterile plastic microfuge vial and quickly frozen at -20°C or at -80°C if DNA is not extracted immediately. The sample should remain frozen until processed, since freeze thaw cycles will release nucleases, which will degrade all RNA and DNA.

Advantages of PCR:

- PCR can be performed on a very small amount of tissue and almost any tissue or body fluid can be used.
- The sensitivity of the technique is high. Generally PCR can detect 10-100 viral genomes which is less than the amount of genomes which is required to form 1 plaque in viral culture.⁷

Pitfalls of PCR:

- Very high sensitivity of PCR has made it more prone to false positive results. This can be discussed as follows^{3,7}
 - o The chance of laboratory contamination is a major risk. PCR can even detect microorganisms shed from the laboratory personals.
 - o PCR can detect DNA of dead colonising normal flora of the conjunctiva.

However these problems can be to some extent overcome with the advent of newer techniques such as real time PCR.
- Again high specificity can give rise to false-negative results, if the target DNA location of the pathogen is pleomorphic.
- PCR cannot detect the organism for which primers have not been provided. So a narrow and well defined differential diagnosis is required for PCR to be effectively useful.

PCR and Koch's postulates^{6,7}

Koch's postulates include isolation of suspected pathogen from all cases of a disease, successful culture of the pathogen in vitro, reinoculation of the pathogen in a host to cause the disease and reisolation of the pathogen from the host. Thus briefly Koch's postulates link an organism to a disease. Though by isolating the organism PCR plays an essential boon in diagnosis of a disease, There are instances (discussed later) where pathogens have been detected by PCR; even they could not be successfully cultured. So, PCR is sometimes utilised for linking a disease to a pathogen, which is not supported by Koch's postulates.

Different methods of PCR:

What is Real time PCR?

This technique of PCR is used to quantify the amount of genomes of a pathogen in a given sample. Low level of genomes of a pathogen in a given sample may indicate decreased presence of that particular pathogen. Thus this may help to differentiate between active infection and latent infections to quantify the amount of pathogens. PCR is performed in a thermocycler provided with real time fluorescence detection unit in each well. In molecular biology it is also known as quantitative real time polymerase chain reaction (Q-PCR/q PCR) or kinetic polymerase chain reaction. So, Real time PCR monitors the fluorescence emitted during the reaction at each PCR cycle in real time as opposed to endpoint detection^{3,4,5,7}.

Real Time PCR

Very simply, real time PCR can be defined as a technique used to monitor the progress of a PCR reaction in real time. Here a fluorescent molecule is used to monitor the PCR as the reaction progresses. The fluorescence emitted by the fluorescent molecule manifolds as the PCR product accumulates with each cycle of amplification.

Basic steps

- Target DNA is amplified in the presence of fluorescein reporter
- Instrument excites and detects fluorescein reporter
- Signal intensity of fluorescein reporter is directly proportional to the amount of amplified DNA

Real time PCR requires a special equipment- light cycler, which is a combination of a thermal cycle and spectrofluorophotometer

Advantages of real time PCR over endpoint PCR:

- Simple
- Relatively quick, as electrophoresis is not required.
- Improved Sensitivity & Specificity
- Permits quantification

Reverse transcriptase PCR:

PCR can be used to detect specific RNAs by converting RNA to DNA with the help of the reverse transcriptase enzyme. The DNA thus produced is known as complementary DNA (cDNA). This cDNA is amplified to the target sequences with the help of PCR and this technique of PCR is known as reverse transcriptase polymerase chain reaction.

Multiplex PCR

PCR reactions can be devised in such a way where multiple primer sets are combined to test for multiple pathogens simultaneously.

Nested PCR:

It involves two sets of primers, used in two successive runs of polymerase chain reaction, the second set intended to amplify a secondary target within the first run product.

Current application of PCR in Ophthalmology:

The first application of PCR in ophthalmology was used in the diagnosis of viral uveitis. Since then, with the advent of newer technique like real time PCR, the role of PCR in modern ophthalmology practice is extensive.

Diagnosis of anterior segment disorders:

The main use of PCR in the diagnosis of anterior segment disorder remains the detection of viral pathogens like herpes family of viruses. PCR has been shown to be useful in detection and serotyping of viral genomes in patients with adenoviral infections. Takeuchi *et al*⁸ discovered a new serotype of adenovirus associated with keratoconjunctivitis outbreak in Japan.

Real time PCR and other nucleic acid amplification tests have been proven to be most sensitive tools in the diagnosis of *chlamydia* infections. These tests can detect as few as one organism per assay, on the other hand presence of 10 organisms are required in sample for detection by conventional methods.⁹ Goldschmidt *et al*⁹ showed that with the help of broad range real time PCR assay, diagnosis of most of the *chlamydia* species is possible with added advantages like reproducibility and rapidity.

Laboratory diagnosis of conditions like ocular tularemia was difficult in pre PCR era. Isolation rate of the organism was only 5% due to fastidious nature of the organism and delay in appearance of specific antibody till 14th day of the diseases. Kantardjiev *et al*¹⁰ has performed PCR assays with the help of conjunctival swab and found it to be a very effective tool in the clinical diagnosis & proper treatment of ocular tularemia and associated conditions like Perinaud syndrome.

Human herpes viruses can widely affect eye and ocular adnexa and their viral genome can be detected by PCR techniques. Though PCR has manifold advantages in detection of HSV DNA, its high sensitivity to detect other nonpathological viruses or latent infections and primer dependent sensitivity had made it a auxiliary tool in the diagnosis of herpes viral diseases. Real time technique has changed this scenario drastically. Removal of the drawback of endpoint PCR & ability to quantify the viral DNA has made the real time PCR an ideal boon in the management of herpetic diseases. Hasegawa *et al*¹¹ with the help of real time PCR technique analysed 144 samples from 90 patients for HSV DNA. They have measured the HSV viral load in various ocular specimens and evaluated the possible viral involvement in various ocular inflammatory diseases of anterior segment. They concluded that in cases with $>10^4$ copies, the result of real

time PCR can be used to reliably diagnose herpetic keratitis and in cases with low copy numbers, diagnosis based on the real time PCR is not recommended.

Acanthamoeba keratitis, though rare, is a cause of fulminant keratitis with devastating clinical sequelae. Low sensitivity to staining with calcofluor white & difficulty in culture often make the diagnosis of acanthamoeba challenging, which in turn seriously jeopardises the management of keratitis. Lehmann *et al*¹² demonstrated 84% and 66% sensitivity of the PCR technique in diagnosis of acanthamoeba from epithelial scrapings and tear respectively in comparison to 53% sensitivity of traditional diagnostic methods. Mathers *et al*¹³ detected acanthamoeba DNA in 24 of 33 specimens with the help of PCR technique.

Diagnosis of posterior segment disorders:

PCR has been proven more than 90% sensitive for detection of VZV, HSV & CMV.^{14, 15, 16} Knox *et al*¹⁷ carried out PCR on 38 eyes of 37 patients of posterior uveitis with diagnostic dilemmas and it has been shown in their study that a definitive diagnosis of CMV, HSV or VZV could be made with the help of PCR in 24 cases. Siqueira *et al*¹⁸ detected CMV genome in aqueous humor in HIV patients with active retinitis and demonstrated that PCR is highly specific in detecting such viral infections.

PCR has also been tried for detection of ocular toxoplasmosis. Initially sensitivity of PCR alone in the diagnosis of ocular toxoplasmosis was 40-60%. The use of intraocular antibody titre along with PCR yields higher sensitivity. Aouizerate *et al*¹⁹ showed that the PCR combined with the determination of the Witmer-Desmonts coefficient (the ratio of aqueous and serum antibodies of toxoplasma) improves the probability of diagnosing ocular toxoplasmosis with a sensitivity up to 72%. However with the help of highly repeated B1 gene of the parasite, Montoya *et al*²⁰ were able to detect toxoplasma DNA in 80% cases of suspected ocular toxoplasmosis. Bou *et al*²¹ showed that the same immuno assay can be used to detect *Toxoplasma gondii* DNA in the peripheral blood of patient with active toxoplasmosis. Mahalakshmi *et al*²² showed a positive PCR result in 51.9% cases with clinically suspected ocular toxoplasmosis which was not significantly less than Witmer-Desmonts coefficient (72.7%). PCR along with restriction fragment length polymorphism analysis has been used to discover three antigenically identical strains of the *Toxoplasma gondii*, which can be considered as an important milestone in the diagnosis & management of ocular toxoplasmosis.²³

Although culture is considered as 'gold standard' in microbiological assessment of the diseases, endophthalmitis vitrectomy study has shown that 30% of cases of endophthalmitis were culture negative. PCR using 16S ribosomal primer (all bacteria share common & highly repetitive DNA sequences for their 16S ribosomal RNA) yields faster result and was studied by Theresa *et al*²⁴ for culture negative cases of endophthalmitis. Bacterial cause of endophthalmitis was noted in 100% of culture positive cases and 44% of culture negative cases. Remaining one third of culture negative cases were found to be fungal. Chiquet *et al*²⁵ analysed aqueous humor samples of 30 patients with post cataract endophthalmitis where 32 % of these cases were culture positive and 61% were positive for eubacterial PCR amplification. However using culture and PCR combination, diagnosis could be made in 71% of cases.

PCR has been proven to be a valuable tool for guiding the management of the endophthalmitis. Intravitreal antibiotic agents used for the treatment of bacterial endophthalmitis are largely dependent on the gram stain status of the bacteria. Carroll *et al*²⁶ used a two step approach to determine the gram stain status of bacteria in endophthalmitis patients. Performing restriction endonuclease digestion of the panbacterial product, Okhravi *et al*²⁷ distinguished 14 different species of endophthalmitis causing bacteria.

The diagnosis of organisms causing delayed onset endophthalmitis is a great challenge as often the organisms are found in lower numbers thereby making them difficult to culture. Lohmann *et al*²⁸ in their study of 25 patients with delayed onset endophthalmitis detected DNA of *Propionibacterium acnes*, *Staphylococcus epidermidis* and *Actinomyces israelii* in 84% and 92% of aqueous sample and vitreous sample respectively. Palani *et al*²⁹ with the help of PCR based restriction fragment length polymorphism analysis identified nontuberculous mycobacteria in three cases of delayed onset endophthalmitis.

PCR has been proven to be a rapid & sensitive diagnostic tool in the diagnosis of fungal endophthalmitis also. Anand *et al*³⁰ studied 43 intraocular specimens from 30 patients with suspected fungal endophthalmitis, 32 of them were found to be positive by PCR including all but 1 of the 24 samples, that were diagnosed as fungal origin by conventional mycologic methods. Biswas *et al*³¹ demonstrated *Aspergillus fumigatus* fungus from paraffin section of an eyeball removed for endogenous endophthalmitis of a 8 month old child.

PCR has been implicated in diagnosis of infectious aetiology of retinal vasculitis also. Madhavan *et al*³² reviewed their experience using PCR to tissue sections obtained from formalin-fixed and paraffin embedded tissues of epiretinal membrane from 23 patients of Eales' disease. 11 out of 23 (47.8%) were positive for *Mycobacterium tuberculosis* genome, indicating association of this bacterium with Eales' disease. Gupta A *et al*³³ reported tubercular retinal vasculitis with varied fundus findings, and diagnosis was confirmed by doing PCR from the aqueous or vitreous humor.

Diagnosis of new infectious association of diseases:

Quentin and Reiber³⁴ showed that patients with Fuch's heterochromic cyclitis had raised intraocular antibody titre & positive RT PCR for rubella virus. Similarly Chee *et al*³⁵ showed that 36% of their patients with either Posner-Schlossman syndrome or Fuch's heterochromic iridocyclitis had positive CMV PCR. Relman *et al*³⁶ using ribosomal 16S primer performed PCR on jejunal biopsy of patients with Whipple's disease and discovered a bacterium related to *Actinomyces-Tropheryma whippelii*, which was isolated in ocular specimens of patients with whipples disease related uveitis by Rickman *et al*³⁷ also. Thus PCR has a great potentiality in establishing associations of pathogens to specific disease and it can be utilised to testify various hypotheses regarding infectious aetiology of various diseases.

Diagnosis of drug resistance:

PCR has been tried to identify the drug resistance strains of viruses also. Cytomegaloviruses have been seen to be associated with higher rates of resistance to common antiviral agents mainly ganciclovir. Ganciclovir resistance in CMV has been found to be due to UL97 gene mutations. Liu *et al*³⁸ performed PCR on 11 vitreous samples of clinically resistant cases of CMV retinitis and drug resistance mutations were detected in 6 cases.

Diagnosis of Masquerade syndromes:

B-cell lymphoma often can mimics a chronic posterior uveitis and lymphoma cells in vitreous and subretinal space may show chronic vitritis and subretinal infiltrates. However using PCR to detect B cell monoclonality of immunoglobulin heavy chain I_H gene rearrangement is highly sensitive.³⁹ Coupland *et al*⁴⁰ showed that PCR can be performed to detect I_H gene rearrangement with the help of chorioretinal biopsy with a high sensitivity.

HLA typing and role in noninfectious uveitis

Polymerase chain reaction-restriction fragment length polymorphism methodology is applied to HLA-DR, -DQ and -DW typing at the nucleotide level, eliminating the need for radioisotopes as well as allele specific oligonucleotide probes.⁴¹ Using this technique, Shino et al reported complete association of the HLA-DRB104 and -DQB104 alleles with Vogt-Koyanagi-Harada (VKH) disease.⁴² Evaluation of intraocular cytokines and other inflammatory mediators and markers provides important information, particularly in noninfectious uveitis.⁴³

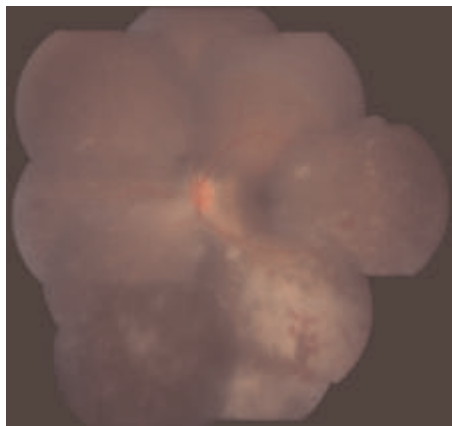
Conclusions

Being a simple, rapid, sensitive and specific technique, it has become a useful adjunct to the existing diagnostic procedure in the field of modern ophthalmology. With the invent of newer techniques such as multiplex, real-time quantification etc PCR has become a powerful tool in molecular technology for evaluation of very small amounts of DNA and RNA.

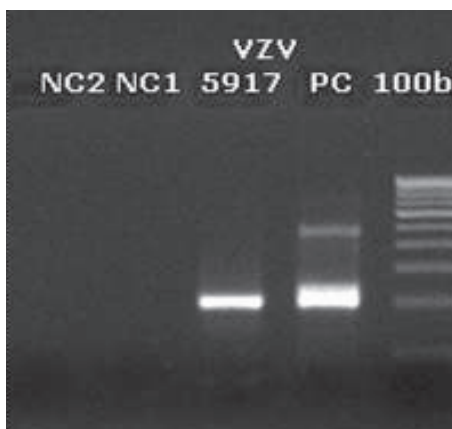
Case Scenarios

Case Scenario 1:

A 28 years young male presented with sudden dimness of vision. On examination, his best corrected visual acuity was 6/6, N6 & 6/36, N18 in right & left eye respectively. Slit lamp examination of the right eye was within normal limit and left eye showed presence of cells in anterior vitreous. Fundus examination of the right eye was within normal limit. Fundus examination of the left showed a hyperaemic disc, scattered necrotizing retinitis & haemorrhages inferiorly. His aqueous aspirate was sent for PCR analysis.

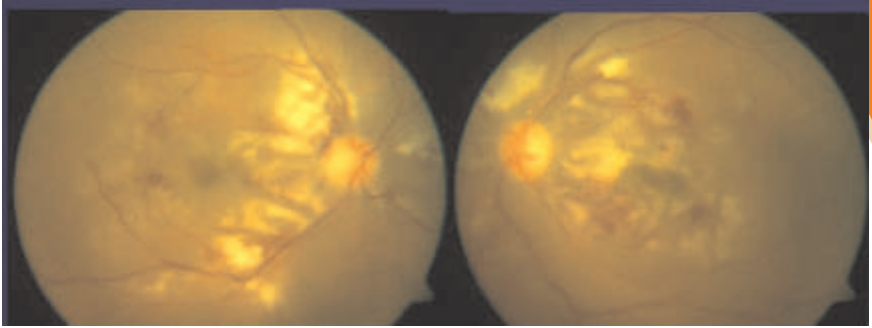


Agarose gel Electrophoretogram showing the results of seminested PCR targeting ORF 63 gene of Varicella Zoster Virus [Lane 1: Negative control 2 Lane 2 : Negative Control 1 Lane 3 AC tap (Lab .no 5917) positive (102 bp amplified product) Lane 4 Positive control (Oka vaccine strain DNA) –Positive (102 bp amplified product) Lane 5 Molecular weight marker 100bp ladder]

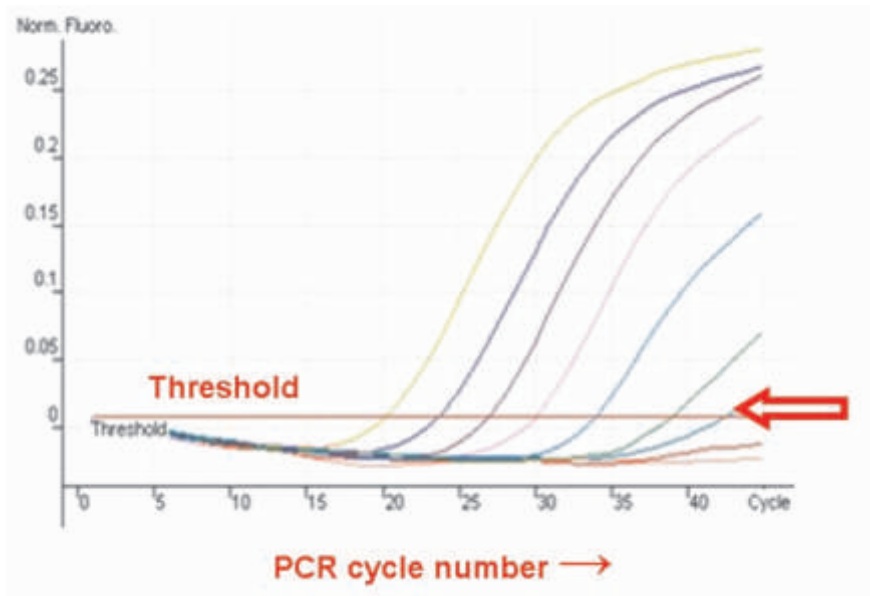


Case Scenario 2:

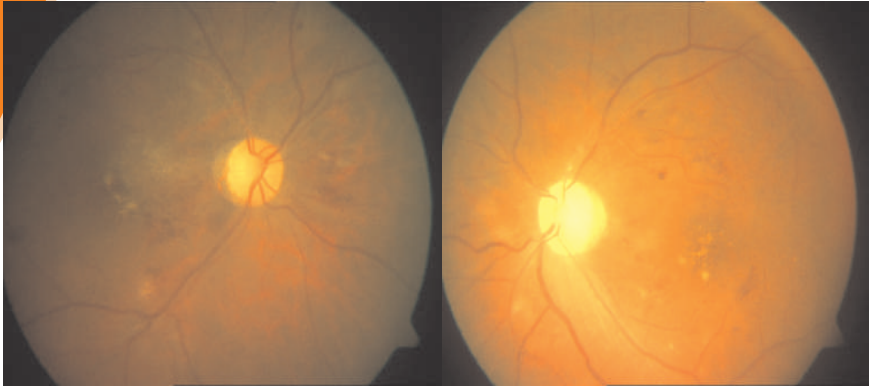
A 48 years male came with complaints of gradual, progressive diminution of vision in both eyes since 15 days. He gave us history of chikungunya fever 15 days before the onset of eye problems. He had consulted elsewhere and was diagnosed as neuroretinitis. His best corrected visual acuity was



2/60, <N36 in right eye and counting finger at 3 meters distance, <N36. Slitlamp examination of both the eyes were normal. Fundus examination of both the eyes showed areas of necrotizing retinitis, haemorrhages. He was started on antiviral treatment with the suspicion of atypical viral retinitis. His aqueous aspirate was sent for PCR analysis, which was negative for HSV, HZV & CMV. He was advised to undergo RT PCR for chikungunya virus



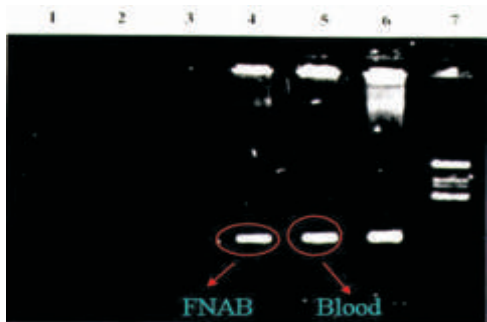
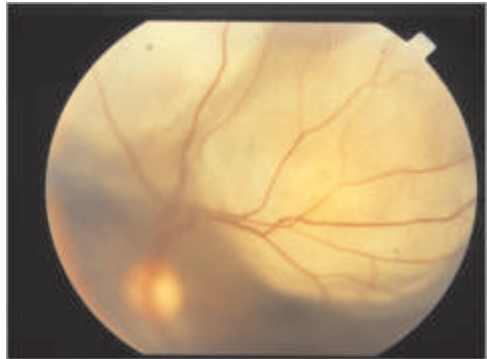
RT PCR for chikungunya virus detected 9 copies of viral RNA. The red arrow showing the copy number of the Chikungunya viral load detected.



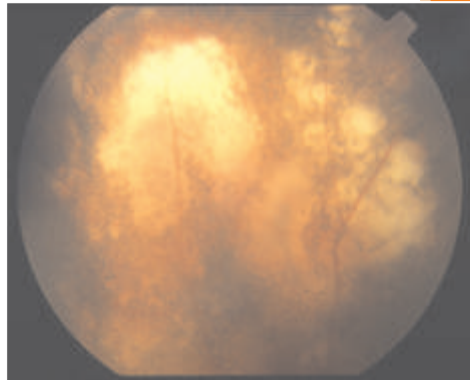
Fundus picture of the same patient, after 2 months of antiviral treatment with oral steroid. His best corrected visual acuity was 6/18, N18 in right eye and 6/24, N18 in left eye.

Case Scenarios 3:

A 14 year old girl came with the complaints of sudden diminution of vision in left eye. Her best corrected visual acuity was 6/6, N6 in right eye and 6/60, N36 in left eye. Slit lamp examination of the left eye showed 2+ Cells, 1+ Flare in anterior chamber, clear lens and plenty of cells in anterior vitreous. Fundus examination of the left eye showed subretinal abscess located superiorly which was about 15 disc diameters in size with an associated exudative retinal detachment. Laboratory investigation revealed high (80 mm) ESR, Negative mantoux (8x 8 mm induration). Chest radiograph showed presence of few calcified hilar lymph nodes. Fine Needle Aspiration biopsy was done and sample was sent for PCR analysis.



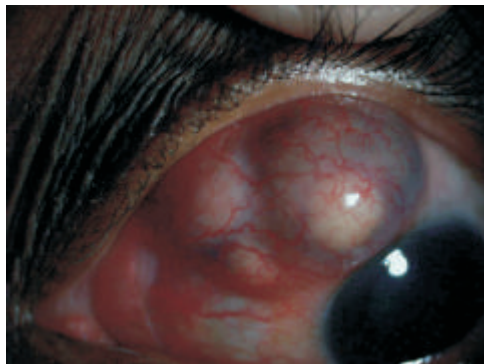
Agarose gel Electrophoretogram showing the results of nested PCR targeting MPB 64 gene of Mycobacterium tuberculosis [Ethidium bromide stained 2% agarose gel with amplification products from case 3 Lane 1: Negative control(Reagent control of the second round, Lane 2: Reagent control of the first round, Lane 3: Aqueous humour- negative, Lane 4: FNAB specimen: Positive with 200 bp amplified product, Lane 5: Blood positive(200bp amplified product),Lane 6: Positive control M.tuberculosis (H37Rv) 200 bp amplified product, Lane 7: Molecular weight marker : Phi X 174 DNA/Hinf I digest.]



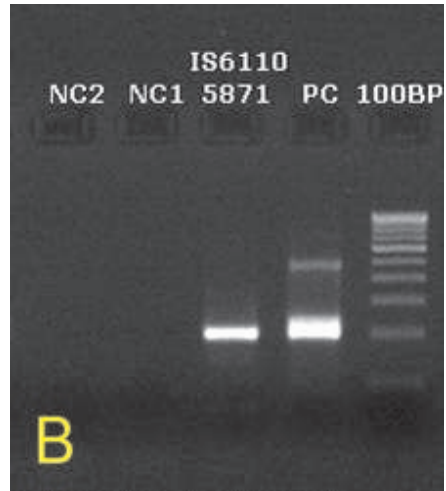
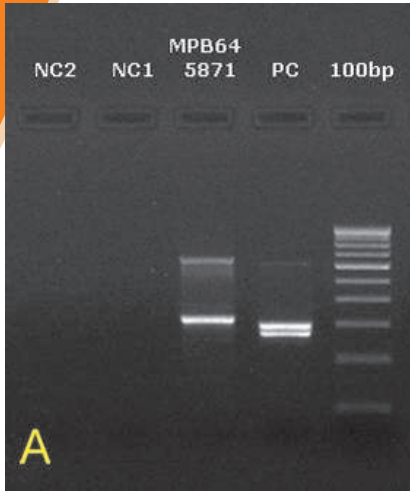
Fundus picture of the same patient after treatment with antitubercular drugs. After completion of 3 months, her best corrected visual acuity was 6/36,N18 in left eye.

Case Scenario 4:

Slit lamp photograph of a 52 year old lady, who presented as a case of nodular scleritis on December, 2009. She was a known case of Rheumatoid arthritis for 6 months. She was treated with Prednisolone, Cyclosporine and Methotrexate by her immunologist. Her condition deteriorated leading on to perforation and was enucleated. Paraffin section of the eye ball was sent for PCR analysis.



Agarose gel Electrophoretogram showing the results of nested PCR targeting MPB 64 gene (FIGURE A) and IS6110 gene (FIGURE B) of Mycobacterium tuberculosis [Fig: Ethidium bromide stained 2% agarose gel with amplification products from case 3 Lane 1: NC2 Negative control (Reagent control) for the second round, Lane 2: NC1 (Reagent control) of



the first round, Lane 3: Aqueous humour(Lab.code No 5871- positive (200bp amplified product), Lane 4: Positive control M. tuberculosis (H37Rv) 200 bp amplified product, Lane 5: Molecular weight marker : 100 bp DNA ladder .]

SUMMARY



- Polymerase chain reaction(PCR) has emerged as a essential powerful rapid laboratory diagnostic technique and a useful adjunct to the conventional gold standard “culture “,with special reference to difficult to grow or slow growing microbial agents like viruses ,Mycobacterium tuberculosis ,anaerobic bacteria or noncultivable microbial agents.
- The results of PCR need to be clinically correlated for treatment purposes as PCR being a highly sensitive technique can detect the DNA of past infection, or dead organisms.
- PCR is an effective tool for associating microbial agents with enigmatic ocular diseases of unknown etiology like Eales disease, or serpiginous choroiditis
- Realtime PCR is a powerful tool for monitoring antiviral therapy in response to antiviral therapy as viral load can be periodically estimated. In addition antibiotic resistance also can be detected by targeting the genes responsible for drug resistance without growing the microbial agents on culture media.
- The rapid, reliable and ultimate diagnostic tool is the PCR based DNA sequencing technique for detection, identification (to species or strain level)and antibiotic resistance of microbial agents associated with ocular infections.

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