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Has the polymerase chain reaction come of age for ophthalmology?

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I vividly remember the week the paper describing the modern polymerase chain reaction (PCR) was published in 1988 [1]. I was working on my PhD thesis, and this technique was a godsend. For the first time we had a biochemical means for generating analytic amounts of DNA from infinitesimal starting quantities, one which did not involve growing prodigious quantities of bacteria or yeast. In those early days, PCR was tedious work – three hours sitting at the bench, transferring tubes between three heat blocks kept at different temperatures, adding enzymes each round. Within a year the advent of automated thermal cyclers and commercial availability of thermostable DNA polymerase turned this into one of the easiest techniques in molecular biology. Performing PCR became so easy it was the first task given to new undergraduates joining our group.

To perform the technique, one needs material containing the DNA to be amplified (i.e. a vitreous, aqueous, or tissue biopsy), some knowledge of the sequence to be amplified (captured as the oligonucleotide primers used to initiate replication), a thermostable DNA polymerase, DNA building blocks (deoxynucleotide triphosphates), a buffer with appropriate salts, a thermal cycler, and a few hours. The phenomenal specificity of DNA binding to its complementary sequence allows the primers to rapidly find their cognate sequences in the template and initiate DNA replication. After synthesis of the daughter DNA strand, heat is used to separate parent and daughter strands, and each becomes template for the next round of amplification. PCR is thus an exponential process. After 30 cycles of PCR, one template molecule may be amplified over a billion times.

This makes PCR ideal for the detection of DNA belonging to a potential pathogen in a complex mixture. Soon after its description, PCR was being used for this purpose. Dlugosch et al. used the technique to detect herpes virus DNA in 1991[2]; PCR assays for a variety of viruses, parasites, bacteria, and fungi affecting the eye have followed over the next decade [3]. Research uses of PCR have included discovery of the organisms underlying several uveitic syndromes, including *Tropheryma whippelii* underlying ocular Whipple disease and *Bartonella henselae*, responsible for some cases of stellate neuroretinitis. Most recently, PCR has been used to describe novel infections in the eye including algae [4] and novel herpes family viruses [5, 6]; viral DNA triggering periocular tumors such as Kaposi sarcoma [7] and Merckel tumor [8]; and ascribing viral causes to syndromes including Fuchs heterochromic

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cyclitis [9] and Posner-Schlossman syndrome [10]. Advances in the last decade have included the ability to test for multiple pathogens simultaneously (multiplex PCR) and the ability to perform quantitative PCR to determine pathogen load.

Despite these prodigious achievements, PCR is not commonly used in clinical ophthalmology practice. Infectious uveitis is relatively rare, so need for any microbiologic technique is not very high. Many infectious forms of uveitis can be diagnosed by clinical examination alone, such as CMV retinitis and ocular toxoplasmosis. In other cases, treatment decision may not be affected by biopsy – for example, most cases of acute retinal necrosis (ARN) syndrome will be treated with acyclovir-family antiviral medications, irrespective of the species of responsible herpes virus.

However, most ophthalmologists will eventually encounter the diagnostic dilemma, where a broad differential diagnosis (such as toxoplasmosis vs. acute retinal necrosis) exists. While it has been clear for many years that PCR can be applied to such cases, it has been less clear until recently that it is truly useful. Some of the difficulty in ascertaining the utility of PCR is due to the dearth of gold standards techniques to establish sensitivity and specificity benchmarks for the technique – for example, what is one to make of a positive PCR for herpes virus with negative cultures, when PCR is more sensitive than culture for detection of pathogen, but also more prone to false-positive results?

Part is also due to the lack of published work to guide its application. Early work by Knox et al. assessed the utility of PCR in diagnostic dilemmas [11]. This group found that PCR was able to detect viral pathogens CMV, VZV, or HSV from 24 of 36 tested vitreous samples from patients with active retinitis. Of the PCR-negative samples in this study, none had clinical courses consistent with a viral retinitis, suggesting substantial clinical utility to PCR testing. Much of the more recent literature on the utility of diagnostic testing of intraocular fluids comes from the excellent work being done in the Netherlands [12-14]. This group has characterized the relative merits of aqueous PCR and intraocular antibody testing (Goldmann-Witmer coefficient) in determining diagnosis of immunocompromised and immunocompetent patients with uveitis. They find that the two approaches are complementary; for example, in the immunocompromised population, PCR is superior for detection of viral infection while intraocular antibody assays are superior for diagnosis of toxoplasmosis. This group has also estimated the clinical impact of intraocular fluid diagnostics. In a study of 152 patients with posterior uveitis of many forms, 29% had either positive PCR or positive intraocular antibody results. Importantly, the authors found that as a consequence of testing, treatment was altered in 24% of patients, suggesting that aqueous testing can have a substantial impact on clinical management.

In the current issue of AJO, Harper et al.[15] publish their retrospective analysis examining the results of PCR testing on 133 patients with possible chorioretinitis. This study is notable for several reasons. First, the authors used a commercial reference laboratory for testing. Most ophthalmologists do not have ready access to a PCR laboratory within their offices and the range of testing available to many community hospital laboratories may be limited. The testing technology used in this study is available to any ophthalmologist with access to an overnight courier. Second, the authors applied PCR testing in ‘real world’ conditions – i.e. when a patient presented with what was thought to be a diagnostic dilemma. While the study might have been improved by a more rigorous definition of ‘diagnostic dilemma’, this work does give a sense of real-world performance of PCR in field conditions. Aqueous was sampled preferentially, as it would be in most ophthalmologists’ offices. The authors ran a total of 433 PCR assays on acquired aqueous and vitreous samples. They found a potential pathogen in 81% of patients by PCR. Perhaps most importantly, treatment of 26 of the 133 patients was altered on the basis of either PCR results or syphilis serology, resulting in

resolution of the ocular inflammation in 25 of the 26 patients. Similar to the results found by the Dutch group, this study suggests that PCR could potentially alter course favorably in nearly ¼ of patients with apparent infectious retinitis or choroiditis.

So, in this, the 21st birthday year of the polymerase chain reaction, it is legitimate to ask if the technique has come of age for routine clinical use – should all cases of chorioretinitis undergo aqueous or vitreous tap and PCR analysis? Because of the potential morbidity in acquiring aqueous and vitreous samples, the answer is still probably not – however, the successes of PCR in identifying organisms that can impact treatment should lower our threshold for utilizing obtaining intraocular fluid. Use of the technique should not be limited to the academic subspecialist; with availability of reference laboratory testing, the community comprehensive ophthalmologist can test for CMV in glaucomatocyclitic crisis as readily as the subspecialist. As the technology is still in active evolution (with the promise of pan-microbial chips capable of diagnosing any known viral or microbial infection from a single sample becoming a reality), PCR is here to stay. Knowledge and facility with the technique will become a necessary part of the ophthalmologist's armamentarium for the foreseeable future.

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