

TOPICS IN Ocular Antiinfectives

Polymerase Chain Reaction (PCR) in Ophthalmology

Janet L. Davis, MD, MA

PCR-based pathogen detection can play a unique role in ophthalmology. In the race to preserve delicate ocular tissue from infection, rapid pathogen identification can be invaluable.

Pathogen identification is an essential first step in managing many types of ocular infection. While presumptive diagnosis may be sufficient for managing superficial ocular infections, infections that breach the cornea or involve sterile intraocular tissues or spaces present formidable challenges. Such infections may be caused by unusual and/or resistant pathogens derived from trauma or local or hematogenous spread.¹ In order to prevent damage to ocular tissue, a search for specific etiologic agents is worthwhile, especially if identification can be made promptly, so that optimal treatment can be given.

Pathogen identification by polymerase chain reaction (PCR) can reduce

the lag time before proper treatment, in some instances allowing for same day initiation of appropriate therapy. In addition, modern molecular techniques, such as PCR, may offer better reliability, reproducibility, and convenience than routine culture techniques. As technology continues to advance, PCR will become an increasingly accessible and cost-efficient adjunct to standard microbiologic culture.

PCR

PCR is a means of detecting specific sequences of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) in a specimen; in microbiology, PCR involves detection of a known sequence of nucleic acids from a unique part of the microorganism's genetic code.

PCR works by placing a clinical sample (eg, aqueous or vitreous fluid) in a thermal cycling machine with the following: a pair of synthetic oligonucleotide primers (designed to align with a target bacterial gene sequence);



FIGURE 1 A PCR thermocycler with real-time capabilities. Reagents and samples are added to the unit on the right. The data is displayed in “real time” on the screen at the left. The number of cycles necessary to achieve an amplification product is especially important, as this indicates the amount of DNA or RNA in the specimen. (Image courtesy of Janet L. Davis, MD, MA.)

a thermostable polymerase enzyme (the “engine” that reproduces or *amplifies* the gene); and a buffered solution of

See INSIDE for: Infectious Scleritis: A Review and Update

by Anat Galor, MD

TARGET AUDIENCE This educational activity is intended for ophthalmologists and ophthalmologists in residency or fellowship training.

LEARNING OBJECTIVES Upon completion of this activity, participants will be able to:

1. Define a role for PCR within their own practices.
2. Describe the types of PCR in current use.
3. Identify clues that should raise suspicion of infectious scleritis.
4. Discuss when to obtain a culture of conjunctival or corneal scrapings in cases of potential infectious scleritis.

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nucleotide triphosphates (the building blocks of the new DNA chain). The system is subjected to cycles of heating and cooling, causing *denaturation* (of the specimen DNA), *annealing* (of primer to the denatured DNA), and *extension* of the template DNA. Each newly generated DNA sequence serves as a template for subsequent cycles; thus expansion proceeds logarithmically until a volume sufficient for visual detection on an electrophoretic gel is reached.²

Types of PCR

PCR was first described in the 1980s and perfected in the 1990s.² Since then, various types of PCR have been developed for microorganism detection. For

example, *universal primer* PCR targets a region of ribosomal RNA that is conserved across large groups of prokaryotic cells; it detects the presence of bacteria or fungi, but cannot speciate or further characterize sample contents. *Multiplex* PCR uses specific primers for multiple organisms in the same reaction mixture. *Reverse transcriptase* PCR (RT-PCR) specifically detects RNA, thus differentiating actively proliferating organisms from inactive ones. Like RT-PCR, *real-time* PCR of DNA or RNA measures the amount of genetic material present in the specimen by comparison to a calibration curve; a clinically significant threshold is determined for eye fluid for specific genes. *Nested* PCR (nPCR)

involves the use of two separate sets of primers targeting a single pathogen in order to boost sensitivity and specificity beyond single-primer technology. *Inverse* PCR takes advantage of the function of restriction enzymes to amplify an unknown sequence positioned adjacent to a known sequence.¹

PCR offers several advantages over conventional techniques. The use of unique primers makes PCR highly specific. It is also highly sensitive, as it is able to detect and amplify minuscule amounts of target material in small volumes of sample, something that is particularly important in ophthalmology. DNA resists degradation, even in suboptimal situations. This is illustrated

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STATEMENT OF NEED

Ophthalmologists face numerous challenges in optimizing their competencies and clinical practices in the realm of preventing, diagnosing, and treating ocular infections and their sequelae; these challenges include:

- The widespread "off-label" use of topical ophthalmic antibiotics to prevent and treat serious and sight-threatening infections—given the reality that the most widely used topical antibiotics in ophthalmology have FDA approvals restricted to bacterial conjunctivitis.
- The escalating levels of multi-drug resistance in common ocular pathogens.¹
- The emergence and increasing prevalence of once-atypical infections that may require diagnostic and treatment techniques relatively unfamiliar to comprehensive ophthalmologists.²
- The introduction of new and potentially more efficacious and/or safe ophthalmic antiinfectives.³
- The introduction of new and potentially more accurate diagnostic techniques for ophthalmic infections.⁴
- Widespread discussion over the efficacy and safety of novel or alternative delivery techniques and vehicles for prophylactic ophthalmic antibiotics (including but not limited to intracameral injection and topical mucoadhesives).^{5,6}
- Increased understanding of the inflammatory damage caused by ocular infections and the best ways to prevent/alleviate inflammation without fueling the growth of pathogenic organisms.

Given the continually evolving challenges described above, *Topics in Ocular Antiinfectives* aims to help ophthalmologists update outdated competencies and narrow gaps between actual and optimal clinical practices. As an ongoing resource, this series will support evidence-based and rational antiinfective choices across a range of ophthalmic clinical situations.

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Internet connection required: Cable modem, DSL, or better.

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by use of PCR for diagnosis and classification of ocular lymphoma when cytology has been rendered unreliable due to poor cellular morphology.³ And, unlike standard culture, PCR can also detect non-cultivable, sparse, or slow-growing organisms, which further augments sensitivity and yield of microbiologic characterization.

As automated techniques improve, PCR will become less labor-intensive, increasingly cost-effective, and more widely available.⁴

PCR Limitations

Despite multiple advantages over other methods, PCR is generally considered adjunctive to standard culture and susceptibility testing. In India, where the prevalence and diversity of ocular infections is high, and reliance on PCR is substantial, PCR is used judiciously. One reason for this is cost—either the cost of maintaining and running automated DNA sequencers in house or the cost of personnel time for obtaining and transporting specimens to an off-site lab. However, costs may be justifiable when diagnostic speed and accuracy provide significant clinical benefit, such as for the identification of mycobacterial, fungal, or other slow-growing pathogens.^{5,6}

PCR is limited to detection of pathogens for which a primer has been developed; typically, a separate test is required for each suspected pathogen. With the development of multiplex PCR and other technologies that incorporate multiple primers significant progress is being made toward mitigating this limitation.^{7,8} Broad spectrum PCR with subsequent DNA sequencing is another strategy to expand detection.

The high sensitivity of PCR can obscure the clinical implications of positive results.² PCR may detect DNA fragments of a non-viable pathogen, or a contaminant, as mentioned above. Such “false positives” may be less of a concern with intraocular specimens compared with other specimen types, however, because of the aseptic conditions under which they are acquired and the inherent sterility of the intraocular compartment in healthy eyes.⁹

A fourth major limitation to patho-

gen detection by PCR is its inability to determine antibiotic susceptibilities, although there are a few exceptions. The genetic code for methicillin resistance among methicillin-resistant *Staphylococcus aureus* (MRSA) is detectable from nasal specimens with 92% sensitivity and 93.5% specificity.¹⁰ Progress is also being made on the identification of triazole- and echinocandin-resistance alleles among fungal pathogens, which, if detected, would impact therapy selection and outcomes.¹¹

A fifth limitation is that, unlike culture, PCR does not allow for archiving of pathogens at research institutions. When using standard culture, clinical isolates can be frozen, stored, and maintained indefinitely in a dormant but revivable form; if further study is needed at later date, specimens can be thawed and analyzed for genotypic and/or phenotypic traits. If standard culture were to be abandoned in favor of culture-independent technologies, our library of ocular pathogens would be diminished.⁴

Current Research

PCR can be used to detect a range of pathogens—viruses, bacteria, fungi, and parasites—obtained from on or within the eye. PCR is an important adjunct to culture for pathogen identification in postoperative endophthalmitis cases, particularly in culture-negative and antibiotic-exposed cases.^{2,12} Bispo and colleagues showed that adjunctive PCR dramatically improved microbiologic detection in aqueous and vitreous samples among patients with bacterial endophthalmitis from 48% (culture only) to 95% (by culture and/or PCR).¹³ PCR is also useful in the workup of delayed-onset endophthalmitis, which is often caused by slow-growing organisms such as fungi (*Aspergillus*, *Candida*, *Fusarium* spp.) or nontuberculous mycobacteria.² PCR can also assist in early detection of delayed-onset endophthalmitis caused by bacteria, including *Propionibacterium acnes*, *Staphylococcus epidermidis*, or *Actinomyces israelii*.^{2,12}

PCR is a highly sensitive and specific tool for determining the cause of infectious uveitis, including detection of herpes simplex virus (HSV), varicella

CORE CONCEPTS

- ▶ PCR works by amplifying nucleic acid sequences until levels are detectable on gel electrophoresis.
- ▶ PCR relies on primers, short segments of synthetic DNA that correspond to a known genetic sequence in a pathogen.
- ▶ PCR may be used to detect viruses, bacteria, fungi, parasites, and mycobacteria.
- ▶ Since it can only detect genetic material and cannot reveal phenotypic properties, PCR is best used as an adjunct to standard culture.
- ▶ For slow growing organisms, PCR can reduce time to pathogen detection from a few weeks to a few days.
- ▶ Patients with endophthalmitis, retinitis, chorioretinitis, uveitis, or keratitis may benefit from PCR testing of ocular specimens for certain suspected pathogens.
- ▶ To learn which PCR tests are currently available consult your clinical microbiology laboratory.

zoster virus (VZV), cytomegalovirus (CMV), Epstein Barr virus (EBV), *Toxoplasma gondii* and *Treponema pallidum*.^{9,14} Patients suspected of having viral retinitis or chorioretinitis, particularly immunocompromised patients with extensive or atypical retinal involvement, may benefit from PCR testing for these pathogens.

For the diagnosis of infectious keratitis—including cases caused by HSV, Chlamydia, bacteria, fungi, and *Acanthamoeba*—PCR is a subject of ongoing interest.^{2,15-17}

Future Applications

Molecular techniques will likely play an increasingly important role in pathogen detection in ophthalmology and, indeed, all of medicine. We are

already seeing more portable instrumentation, expanded primer sets, and more widespread clinical availability of PCR testing. Microbial genome sequencing (and comparing the results with large genome databases) could become a means for outbreak and clinical diagnostic testing.⁴ The ability to use PCR to detect the genetic code behind microbial toxins could prove invaluable in the management of toxin-mediated diseases once anti-toxin therapies find their way into the therapeutic arsenal.

Conclusion

PCR is an appropriate and increasingly important adjunctive diagnostic tool for select cases of ocular infection. Clinicians interested in optimizing use of PCR should consult with their clinical microbiology laboratory to find out which tests are available where they practice and which may be performed at outside laboratories. Protocols for tissue procurement and transport should be followed carefully and periodically updated as the technology evolves.

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Infectious Scleritis: A Review and Update

Anat Galor, MD

Being willing to consider the possibility of an infectious cause could make the difference between saving and losing vision in eyes with scleritis.

Unlike more external ocular structures, the sclera is highly resistant to invasion and infection. This may be due, in part, to an abundance of episcleral vessels in the sclera, which provide a channel for rapid clearing of potential pathogens. Resistance to invasion and infection may also be a function of the sclera's position within the anatomy of the eye: it is relatively removed from the external environment and thus has less contact with foreign bodies such as contact lenses. Regardless of the reason, the sclera is more likely to become inflamed than infected, and autoimmune scleritis is more prevalent than scleritis caused by infection or drug reaction.

The danger in quickly categorizing scleritis as inflammatory/autoimmune is that a serious scleral infection can be overlooked. Though rare, infectious scleritis is a sight-threatening emergency—and the prognosis is poor. Across causes, infectious scleritis is associated with loss of functional vision in about 50% of patients and loss of an eye (due to evisceration or enucleation in order to control the infection and/or eliminate

pain) in about 25%.¹ Because of its severity—and because immunosuppressive therapy directed against a misdiagnosed case of infectious scleritis may worsen the infection—infectious causes must be considered in all scleritis cases.

Patient Presentation

Patients with scleritis of any type typically present with a painful, red eye and nonblanching, immobile blood vessels. Blood vessels that do not blanch with phenylephrine and do not move when touched with a cotton swab are usually deep in the sclera—indicating scleritis—in contrast to superficial vessels in the conjunctiva or episclera that do respond to such tests. Although most scleritis cases are noninfectious, one must look for historical and physical clues consistent with infection once scleritis is suspected (Table 1).

The clinician should obtain a careful history from all patients with scleritis by inquiring about autoimmune disorders as well as risk factors for infectious scleritis, including a history of ocular surgery (recent or remote), ocular trauma (penetrating or nonpenetrating), and immune suppression. Delayed infectious scleritis has been reported months to years following pterygium surgery. In a recent retrospective review of infectious scleritis cases between 1987 and 2010 at Bascom Palmer, our group found that,

CORE CONCEPTS

- Scleral infection is rare but frequently devastating.
- Necrosis may be associated with immune or infectious scleritis.
- Necrosis warrants immediate referral to a facility equipped to evaluate and manage ophthalmic emergencies.
- All patients with suspected infectious scleritis should undergo comprehensive culture and aggressive treatment.
- *Pseudomonas* is the most common culprit in the US.
- Prognosis is generally poor and relates to presenting visual acuity.
- The armamentarium against infectious keratitis is expanding with novel surgical and medication delivery technologies.

among patients with a history of pterygium surgery, median time from inciting event to scleritis presentation was 49 months (range 0 to 183 months).¹ This is far longer than the median time from event to symptoms across all causes—1.9 months—in the same study.

Immunosuppression related to HIV/AIDS, cancer chemotherapy, and bone marrow transplant (among several other causes) also increases risk for infectious scleritis.²

Since infectious scleritis is rare, epidemiologic trends are not known; however, incidence may be decreasing as surgical techniques improve and risk factors are less commonly experienced. Technologic advances have allowed for a reduction in aggressive radiation and mitomycin C use with pterygium removal, insults which have been associ-

TABLE 1

Historical and physical findings that should prompt consideration of infectious scleritis in the patient with a painful, red eye

| History | Clinical signs or course |
|--|--|
| Surgery, particularly pterygium (remote or recent) | Necrosis |
| Trauma | Abscess* |
| Systemic immunosuppression | Involvement of adjacent structures (eg, keratitis, endophthalmitis)* |
| | Not improving on immunosuppressive therapy |

*Highly suggestive of infectious cause

ated with progressive change to scleral anatomy and increased infection risk.^{2,3}

Disease Detection

Examination of the eye may reveal signs of infection or possible infection. The presence of an abscess, multiple microabscesses, or obvious involvement of neighboring structures (eg, endophthalmitis or keratitis) are clues that scleritis is likely infectious in origin.

Another very important clue is necrosis. By itself, necrosis does not distinguish infectious from noninfectious scleritis; however, among cases of infectious scleritis, necrosis is almost universally present. In our series of infectious scleritis patients, necrosis was present among 93%.¹ Necrosis appears upon fluorescein staining as a soupy area within the white of the eye (Figure 1). Necrosis is a medical emergency, as it indicates a melting of the structures of the eye and often results in a poor outcome. In the absence of clear-cut autoimmune history or infectious signs, the presence of necrosis in a patient with

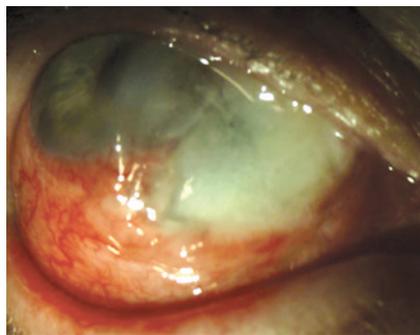


FIGURE 1 Abscess associated with staphylococcal scleritis.

scleritis presents a serious diagnostic dilemma: cultures for the gamut of potential pathogens must be obtained.

Although necrosis is usually present in cases of infectious scleritis, it is not present 100% of the time. Some pathogens, such as *Nocardia*, are less likely to produce necrosis. Thus, it is important to remember that infectious scleritis may present without infectious risk factors or outward signs of infection. Such cases are generally assumed

to be immune-mediated; however, if patients fail to respond to appropriate immunomodulating treatment (eg, nonsteroidal anti-inflammatory drugs followed by high-dose corticosteroids), the possibility of infectious scleritis must be revisited.

Pathogens

Pseudomonas aeruginosa is the most common cause of infectious scleritis, responsible for 85% of cases. Other bacterial pathogens include *Streptococcus*, *Staphylococcus* (including methicillin-resistant *Staphylococcus aureus*), *Haemophilus*, *Enterobacter*, and *Propionibacterium* spp. Amoebic (eg, *Acanthamoeba*) and fungal (eg, *Fusarium*, *Aspergillus*, and *Candida*) pathogens have also been reported as causes of infectious scleritis. Acid-fast *Mycobacterium chelonae* should be suspected in cases of infectious scleritis associated with retinal surgeries, specifically scleral buckle.^{1,2} Viruses including herpes simplex virus (HSV) and varicella zoster virus (VZV) have been reported to cause scleritis.

At our institution, standard pan-bacterial/fungal culture for suspected infectious scleritis includes use of thio-glycollate broth, blood agar, chocolate agar, and Sabouraud's agar. Special tests for rare pathogens include agar culture with *E. coli* overlay to rule out *Acanthamoeba* (eg, in cases associated with corneal ulcer in a contact lens wearer); Lowenstein-Jensen media for mycobacterial culture (eg, in cases associated with history of scleral buckle procedure); and culture, PCR, or direct immunofluorescence for virus detection (eg, in HSV cases).

Prognosis

Vision on presentation is an important indicator of individual prognosis.¹ Patients who present with functional vision, and who are promptly diagnosed and treated, have a reasonable chance of emerging with functional vision intact. By contrast, patients with markedly impaired vision and irreversible, infection-related structural complications at presentation are less likely to

regain functional vision.¹

Concomitant keratitis or endophthalmitis and infection with a fungal pathogen are also associated with poorer outcomes.¹

Management

The appropriate approach to management of infectious scleritis depends on the patient, the pathogen, and the extent of involvement. The first step is to make the correct diagnosis; the second step is to eradicate the infection with a patient-tailored combination of medical and sometimes surgical methods. Medical therapies include topical, systemic, and/or subconjunctival antimicrobial agents directed against suspected or proven pathogens. In addition, debridement and drainage of abscessed or necrotic tissue reduces microbe burden and enables antiinfective therapy to reach its target.

Cryotherapy is a freezing technique that may be used for antiinfective purposes in the treatment of infectious scleritis. Corneal or scleral patch grafting may be therapeutic in the face of perforation or impending perforation, although they must be used cautiously, as they can mask an ongoing infection or inhibit the penetration of antibiotics.

Advanced Therapies

The avascular and tightly bound collagen composition of the cornea and sclera make these structures difficult to treat with topical and systemic antimicrobial therapy.³ Patients who are not improving with conventional therapies may be candidates for advanced or experimental therapies designed to improve penetration of drugs to the site. One such technique involves continuous subconjunctival infusion of an antibiotic, such as tobramycin or levofloxacin, for up to 2 weeks.³ This technique not only administers the antibiotic but also cleanses necrotic debris; it has been associated with successful treatment of scleritis, including cases due to *Pseudomonas*.³

UV-A/riboflavin collagen cross-linking is a technique that changes the biomechanical properties of corneal

collagen, making it stiffer, more stable, and less susceptible to enzymatic degradation by bacteria. Collagen cross-linking was invented to interrupt the progression of keratoconus and has since been applied to a range of ophthalmic conditions including corneal ectasia, Fuchs corneal dystrophy, pseudophakic bullous keratopathy, and infectious keratitis. In addition to its stiffening effects, the UV light used in crosslinking kills bacteria by damaging cell membranes and nucleic acids.⁴ Crosslinking has been successfully used in graft preparation following conjunctival and scleral melting.⁵

Iontophoresis is a novel noninvasive means for enhanced drug delivery into hard-to-reach tissues using a small electric current.⁶ Iontophoresis has been used in various fields of medicine and is in development for ophthalmic use at Bascom Palmer. In animal models of *Pseudomonas* keratitis, iontophoresis with gentamicin-loaded hydrogels have been shown to significantly reduce bac-

terial load compared with conventional topical treatments.⁶

Conclusion

For patients with scleritis, a high index of clinical suspicion for infection and expedient care can mean the difference between functional vision and blindness. Novel treatment approaches and advances in surgical techniques that preserve the integrity of the sclera should improve the overall prognosis of this potentially devastating disease.

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that in the past 12 months, she has not had a financial relationship with any commercial organization that produces, markets, re-sells, or distributes healthcare goods or services consumed by or used on patients. Medical writer Noelle Lake, MD, assisted in the preparation of this article.

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EXAMINATION QUESTIONS TOPICS IN OCULAR ANTIINFECTIVES, ISSUE 55

This CME program is sponsored by the University of Florida College of Medicine and supported by an unrestricted educational grant from Bausch + Lomb, Inc. **DIRECTIONS:** Select the one best answer to each question in the Exam (Questions 1–10) and in the Evaluation (Questions 11–16) below by circling one letter for each answer. Participants must score at least 80% on the questions and complete the entire Evaluation section on the form below. The University of Florida College of Medicine designates this activity for a maximum of 1.0 AMA PRA Category 1 Credit™. There is no fee to participate in this activity. You can take the test online at <http://cme.ufl.edu/ocular>.

- PCR, in its current state, would be well used in which of the following clinical scenarios?
 - Identification of a slow-growing ocular pathogen
 - Determining fluoroquinolone MIC against common ocular pathogens
 - Biochemical characterization of a fungal pathogen
 - All of the above
- Which of the following is NOT a risk factor for the development of infectious scleritis?
 - Pterygium surgery 5 years ago
 - Pterygium surgery 2 weeks ago
 - Current chemotherapy
 - All are risk factors
- Which of the following is directly detectable by PCR?
 - Bacterial membrane
 - Exotoxin
 - DNA or RNA
 - All of the above
- Which of the following potential scleritis therapies was initially developed as a treatment for keratoconus?
 - Iontophoresis
 - Continuous subconjunctival infusion
 - Collagen crosslinking
 - Cryotherapy
- Which of the following may be diagnosed by PCR?
 - Delayed-onset endophthalmitis caused by *Propionibacterium acnes*
 - Acute postoperative endophthalmitis caused by coagulase-negative staphylococcus
 - Posterior uveitis caused by cytomegalovirus
 - All of the above
- How does real-time PCR differ from ordinary PCR?
 - Requires gel electrophoresis
 - Can only be performed on fresh, unfrozen specimens
 - Quantitates amplification after each cycle
 - Used solely for DNA assays
- Which of the following statement(s) is/are true regarding necrosis associated with scleritis?
 - The presence of necrosis rules out noninfectious causes
 - All patients with infectious scleritis develop necrosis
 - Necrosis is an ophthalmic emergency
 - All of the above are correct
- A patient presents with a painful, red eye and nonblanching, nonmobile vessels. What is the most likely diagnosis?
 - Scleritis
 - Episcleritis
 - Keratitis
 - Conjunctivitis
- Which of the following organisms is implicated in a majority of infectious scleritis cases?
 - Acanthamoeba castellanii*
 - Pseudomonas aeruginosa*
 - Mycobacterium chelonae*
 - Serratia marcescens*
- Which of the following factors contribute to the high sensitivity associated with PCR?
 - Primer alignment with known genetic sequences
 - Ability to amplify target from low-volume specimens
 - Ability to detect nonviable organisms
 - All of the above

EXAMINATION ANSWER SHEET TOPICS IN OCULAR ANTIINFECTIVES, ISSUE 55

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ANSWERS:

- | | |
|------------|-------------|
| 1. A B C D | 6. A B C D |
| 2. A B C D | 7. A B C D |
| 3. A B C D | 8. A B C D |
| 4. A B C D | 9. A B C D |
| 5. A B C D | 10. A B C D |

EVALUATION:

1=Poor 2=Fair 3=Satisfactory 4=Good 5=Outstanding

- Extent to which the activity met the identified:
 - Objective 1: 1 2 3 4 5
 - Objective 2: 1 2 3 4 5
 - Objective 3: 1 2 3 4 5
 - Objective 4: 1 2 3 4 5
- Rate the overall effectiveness of how the activity:
 - Related to my practice: 1 2 3 4 5
 - Will influence how I practice: 1 2 3 4 5
 - Will help me improve patient care: 1 2 3 4 5
 - Stimulated my intellectual curiosity: 1 2 3 4 5
 - Overall quality of material: 1 2 3 4 5
 - Overall met my expectations: 1 2 3 4 5
 - Avoided commercial bias/influence: 1 2 3 4 5
- Will the information presented cause you to make any changes in your practice? Yes No
- If yes, please describe: _____
- How committed are you to making these changes? 1 2 3 4 5
- Are future activities on this topic important to you? Yes No

If you wish to receive credit for this activity, please fill in the following information. Retain a copy for your records.

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