

Laboratory support in the diagnosis of uveitis

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Intraocular inflammations are still a diagnostic challenge for ophthalmologists. It is often difficult to make a precise etiological diagnosis in certain situations. Recently, there have been several advances in the investigations of uveitis, which has helped the ophthalmologists a lot in the management of such clinical conditions. A tailored approach to laboratory diagnosis of uveitic cases should be directed by the history, patient's symptoms and signs, and clinical examination. This review summarizes various modalities of laboratory investigations and their role in the diagnosis of uveitis.

Key words: Angiotensin converting enzyme, antitoxoplasma antibody, laboratory test, tuberculin test

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Intraocular inflammations are still a diagnostic challenge for ophthalmologists. Management of these conditions has improved in recent years; still it is often difficult to make a precise etiological diagnosis in certain situations. Recently, there have been several advances in the investigations of uveitis, which has helped the ophthalmologists a lot in the management. Although some diseases are local ocular immune phenomena, many of them are systemic with ocular manifestations. The spectrum of disease pathogenesis ranges from autoimmunity to neoplasm to viruses, so the proper management of uveitis requires an understanding of internal medicine, infectious diseases, rheumatology, and immunology. Approach to laboratory diagnosis of uveitic cases should be directed by the history, patient's symptoms and signs, and clinical examination. In this review, we have tried to summarize various modalities of laboratory investigations and their role in the diagnosis of uveitis.

1. Routine blood investigations

Routine blood investigations like complete blood count (CBC) and erythrocyte sedimentation rate (ESR) often may not yield any specific diagnosis in uveitic cases, but they should be advised in all patients. They often provide a clue to underlying clinical conditions. For example, eosinophilia in a patient can point toward sarcoidosis, parasitic infections, etc. Similarly raised white blood cell count in bacterial infections, relative lymphocytosis in viral infections, or tuberculosis (TB) often help the treating ophthalmologist in planning further investigations. Also information on blood count is of paramount importance, especially when planning

for immunosuppressive treatment. Similarly, peripheral blood smears are helpful to rule out conditions like malignancies of blood. Liver function tests, renal function tests, and blood glucose levels are usually done to establish baseline normal levels and to monitor response to treatment when the patient is on steroids and immunosuppressives.

2. Disease-specific laboratory investigations

Diagnosis of Sarcoidosis

Sarcoidosis is a multisystem granulomatous disease, which commonly involves the lungs, thoracic lymph nodes, the skin, and the eyes. Various laboratory parameters used in the diagnosis of sarcoidosis are discussed below.

Serum angiotensin converting enzyme (ACE) and serum lysozyme are often grouped together as both tests measure the same parameter, i.e., macrophage products produced by the sarcoid granulomas. ACE is an enzyme of the renin-angiotensin pathway, and the normal level in serum is approximately 55 IU/L. The level of serum ACE reflects the total body mass of active sarcoid granulomas. Elevated serum ACE levels are seen in 60%–90% of patients with sarcoidosis.^[1] However, ACE is normally secreted by pulmonary macrophages and vascular endothelium. Therefore, it is not pathognomonic of sarcoidosis and levels may also be raised in various conditions. Raised levels are also found in children, the reason for which it cannot be used for the diagnosis of sarcoidosis in children. In localized sarcoidosis like ocular involvement, ACE secretion by the sarcoid granulomas may be minimal to raise its serum level and a normal ACE level does not exclude the disease. Serum ACE levels have been found to be elevated in the aqueous humor of patients with ocular sarcoidosis.^[2] Serum lysozyme levels may also be raised above its normal limit of 8 mg/L in patients with sarcoidosis. This parameter is helpful in patients on ACE inhibitor, where the exact measurement of serum ACE is not possible. In a study on 125 sarcoidosis cases, this parameter was elevated in 76% of patients, whereas raised serum ACE was found to be elevated in 60% of cases.^[3] Kawaguchi *et al.* in their

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study of biopsy-proven cases of sarcoidosis have reported that the combined sensitivity, specificity, and positive and negative predictive values of raised serum lysozyme in diagnosis of sarcoidosis are better than raised serum ACE levels.^[4]

In developing countries where Bacilli-Calmette-Guerin (BCG) vaccination is routinely performed, negative tuberculin test in a BCG-vaccinated patient or in a patient with a previously positive tuberculin skin test (TST) is of great value in diagnosis of sarcoidosis. This is the most well-known manifestation of anergy. It has been recommended as one of the diagnostic criteria by the first international workshop on ocular sarcoidosis.^[5] Table 1 summarizes the laboratory diagnosis criteria recommended by first international workshop on ocular sarcoidosis.^[5]

Liver is one of the occult sites where sarcoid granuloma can occur and remain undetected. Elevated liver enzymes are of diagnostic value when serum levels of alkaline phosphatase are more than three times the upper limit of normal values or when two of the following three liver enzymes aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase are more than twice the upper limit of normal values.^[6]

As a result of increased calcium absorption after an increased production of 1,25-dihydroxycholecalciferol by the sarcoid granulomas, hypercalcemia and hypercalciuria occur in some patients with sarcoidosis.

Confirmation of the diagnosis of sarcoidosis or diagnosis of definite ocular sarcoidosis can be made by solid-tissue biopsy showing classic noncaseating granulomas, and preferably at more than one site. Skin, conjunctiva, and lacrimal glands are the common sites for nodular lesions in sarcoid. "Blind" conjunctival biopsy, i.e., biopsy where no lesions are seen on examination, though controversial, are advocated by some authors. In a study by Nicholas *et al.*, 55% of patients with biopsy-proven sarcoidosis from other sites, a blind conjunctival biopsy was positive.^[7]

Sarcoidosis frequently involves the lacrimal gland and granulomas were found on biopsy of the lacrimal gland in 22% of patients with presumed ocular sarcoidosis.^[8] Keeping the complications of the procedure in mind, lacrimal gland biopsy can be recommended if there is increased uptake on a gallium scan or in case of clinically enlargement of the gland. Biopsy of the cutaneous nodules also can be helpful. However, it should be kept in mind that erythema nodosum is a nonspecific finding in sarcoidosis and biopsy of such lesions is unjustified.

The historical Kveim test, where antigen prepared from the spleens of patients with proven sarcoidosis was injected

intradermally to a patient with suspected sarcoidosis, is not performed now a day.

Diagnosis of Ocular Tuberculosis

Laboratory diagnosis of ocular TB is quiet difficult and challenging. For definitive diagnosis of ocular TB, in accordance with Koch's postulate, the presence of *Mycobacterium tuberculosis* organisms in ocular tissues or fluids should be demonstrated either histologically or microbiologically. This is often not possible because of difficulty in obtaining tissue samples and justification of performing invasive procedure in patients where clinical diagnosis often overlaps with the other various mimicking conditions. However, with the advent of polymerase chain reaction (PCR), the direct evidence of demonstrating mycobacterial pathogens in ocular tissues and subsequent management has become easier.

TST, popularly known as Mantoux test, is an intradermal test based on the type IV hypersensitivity reaction for the diagnosis of latent TB. A standard dose of 5 tuberculin units (0.1 mL) of purified protein derivative (PPD) is injected intradermally in the volar surface of forearm. PPD is a precipitate of non-species-specific molecules obtained from filtrates of sterilized, concentrated cultures which mainly consists of more than 200 proteins derived from *M. tuberculosis*. The reaction is read after 48–72 h by measuring the diameter of induration (palpable raised hardened area) across the forearm (perpendicular to the long axis) in millimeters. An induration of 10 mm is considered as positive. The test has several limitations like cross-reactivity of the antigen used in the test with BCG and environmental nontuberculous mycobacterias, booster effect on repeated injection of PPD leading to false-positive results. The test is also prone to false-negative results, especially when used in immunocompromised patients. Thus, because of its relatively low sensitivity and specificity and its inability to discriminate between latent infection and active disease, the test is of limited value in the diagnosis of active TB.^[9,10]

A specific genomic region present in the *M. tuberculosis* has been identified which is not present in BCG vaccine, *Mycobacterium bovis*, and most environmental mycobacteria. This region encodes several highly immunogenic antigens which are capable of eliciting vigorous helper T-cell responses in patients with TB. Early secretory antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10) are such antigens, which are used in a newer method of diagnosis of TB known as interferon gamma release assay (IGRA). ESAT-6 and CFP-10 stimulate helper T cell which results in to secretion of interferon gamma [Fig. 1]. IGRAs measure *in vitro* T-cell responses to these antigens by quantification of interferon gamma using an enzyme-linked immunosorbent assay (ELISA) or an enzyme-linked immunospot assay.^[11]

IGRAs are more specific than the TST because of less cross-reactivity to BCG vaccine and nontuberculous mycobacteria. However, ESAT-6 and CFP-10 are found in *M. kansasii*, *M. szulgai*, and *M. marinum* and sensitization to these organisms may release IFN- γ and can give false-positive IGRA results.^[11] ESAT-6 and CFP-10 are recognized by fewer T lymphocytes and hence stimulate less IFN- γ to release; so a more sensitive ELISA than is required to measure IFN- γ concentrations. Fresh blood specimen that contains viable white blood cells is required for this procedure to measure IFN- γ response accurately.

Table 1: Laboratory diagnosis criteria recommended by first international workshop on ocular sarcoidosis

1. Negative tuberculin test in a BCG-vaccinated patient or having had a positive PPD (or Mantoux) skin test previously
2. Elevated serum angiotensin converting enzyme (ACE) and/or elevated serum lysozyme a
3. Chest x-ray; look for bilateral hilar lymphadenopathy (BHL)
4. Abnormal liver enzyme tests (any two of alkaline phosphatase, ASAT, ALAT, LDH, or γ -GT)
5. Chest CT scan in patients with negative chest x-ray

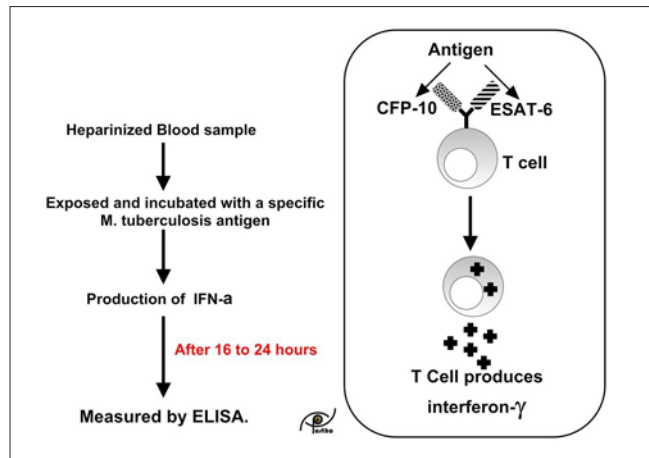


Figure 1: Principles of interferon gamma release assay

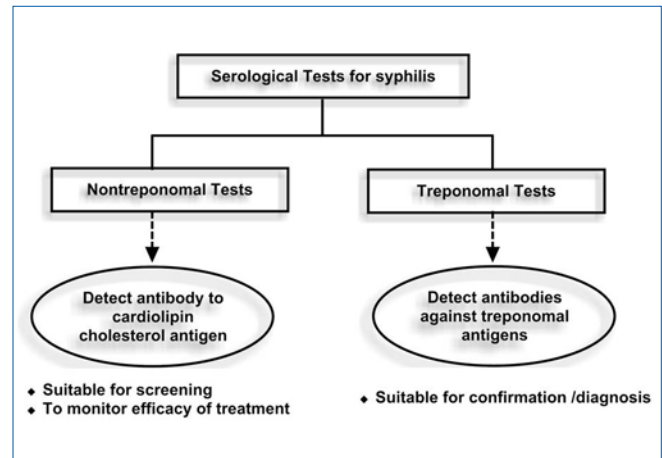


Figure 2: Various tests used in diagnosis of ocular syphilis

Diagnosis of Syphilis

A diagnosis of ocular syphilis is based on a clinical suspicion which can be confirmed by appropriate diagnostic tests. Various types of tests are available for the diagnosis of syphilis [Fig. 2]. Dark field microscopy involves direct identification of *Treponema pallidum* by examining exudates from chancre or condyloma latum with compound microscope with a dark field condenser. In syphilitic infection, there is production of nonspecific antibodies which react to cardiolipin. This forms the basis of traditional nontreponemal tests such as Venereal Disease Research Laboratory (VDRL) tests and rapid plasma regain tests. On the other hand, the treponemal tests like fluorescent treponemal antibody absorption FTA-ABS and microhemagglutination – *T. pallidum* (MHA-TP) assays detect antibodies against *T. pallidum*. These tests are mainly used to confirm the diagnosis of syphilis in a patient with reactive nontreponemal tests. The nontreponemal tests show a decline in titres or become nonreactive with treatment, so they can be used to assess the response to treatment. Treponemal tests generally remain reactive for life. These tests are associated with a lower incidence of false positivity and they are more specific. In some patients with HIV infection, none of the above tests are reactive and can have atypical test results like unusually low or high titres; though in majority of the HIV patients, these serological tests show accurate results. For diagnosis of neurosyphilis, there is no definite test. Though VDRL–CSF is highly specific, it is insensitive. Although, the CSF FTA-ABS is less specific for neurosyphilis than CSF VDRL (i.e., yields more false-positive results), it is highly sensitive. As patients with syphilitic uveitis or other ocular involvement are frequently associated with neurosyphilis, a CSF examination needs to be considered in such patients for confirmation of diagnosis.^[12,13]

Diagnosis of Ocular Toxoplasmosis

Diagnosis of ocular toxoplasmosis is almost always clinical. However, laboratory diagnosis of this clinical entity is of paramount importance in cases of atypical presentations, subclinical infections etc. Also, it must be remembered that many of the serological tests used for diagnosis of toxoplasma infection are often positive in general populations and do not necessitate any active treatment.

The serological diagnosis of ocular toxoplasmosis is confirmed by measurement of intraocular parasite-specific antibody production, which is an indirect proof of the presence of the parasite within the eye. The various serological methods for detecting anti-toxoplasma antibodies include Sabin–Feldman test, the complement fixation test, the agglutination tests, the indirect immunofluorescence assay (IFA), and the ELISA. The Sabin–Feldman dye test though remains the gold standard for the diagnosis of toxoplasmosis, is no longer performed routinely as it requires the constant maintenance of virulent organisms in the laboratory with the associated risk of cross-infection to the laboratory personnel.

An acute *Toxoplasma gondii* infection can be demonstrated by detection of specific IgM or IgA antibodies, or both, in the blood. Immunoglobulin M usually appears in the first week after infection, peaks at 1 month, and disappears after 9 months.^[14] However, when very sensitive assays, such as an immunosorbent agglutination assay, are used, IgM can be detected even after 1 year of infection.^[15] For the detection of congenital toxoplasmosis, IgA antibodies are often used, because the IgM production is still weak in new borns and IgG antibodies can be of maternal origin.^[16] Role of anti-IgA antibodies in the diagnosis of acquired ocular toxoplasmosis has been studied by various authors. IgA antibodies occur early after an acquired *T. gondii* infection and disappear earlier than IgM antibodies. Ongkosuwito *et al.*^[17] used IgA antibodies to study ocular disease in acquired ocular toxoplasmosis. They observed that IgA is a more sensitive measure of acquired infection than standard techniques, although it may be less specific. Ronday *et al.*^[18] found that the addition of IgA testing increased the sensitivity of diagnosis of *T. gondii* infection. Though the presence of anti *T. gondii* IgG antibodies does not confirm the diagnosis, a negative IgG usually discards the possibility. Anti *T. gondii* IgG antibodies can persist at high titers for years after the acute infection and there is a high prevalence of such antibodies in the general population.^[17] Thus, the presence of specific antibodies in the form of cell-mediated immunity in the blood of patients is not discriminatory for ocular disease and may not even be related to the ocular lesion. The demonstration of local synthesis of specific antibodies is a valuable diagnostic tool in such conditions. Intraocular antibody production is considered to be significant if the relative amount of specific antibodies (compared to the total immunoglobulin

level found in the aqueous) exceeds the relative amount of these antibodies in the serum. This intraocular production of antibody can be calculated by the Goldmann-Witmer (GW) coefficient according to the following formula:^[19-21]

GW coefficient = (Intraocular anti-Toxoplasma IgG/ Intraocular IgG) / (Serum anti-Toxoplasma IgG/Serum IgG)

Diagnosis of Ocular Toxocariasis

Toxocariasis is an infection caused by the accidental ingestion of larvae of the dog roundworm *Toxocara canis* or the cat roundworm *Toxocara cati*. Children who have pica and are in close contact with pets are mainly vulnerable. Ocular toxocariasis is diagnosed based on a positive history of contact with pets and suggestive ocular findings. Diagnosis is mainly clinical, although ELISA with *Toxocara* excretory-secretory antigen (TES-Ag) has been shown to be highly specific for toxocara infection. An increase of anti-TES-Ag IgE level indicates acute toxocara infection or progressive inflammation and increase in the IgG level confirms a past or present infection with minimum inflammation.

Diagnosis of Systemic Rheumatic Diseases

Systemic rheumatic diseases are a heterogeneous collection of immune-mediated, multisystem disorders. Ocular involvement is common and varies with the type of systemic rheumatic disease. Laboratory investigations and their role in the diagnosis of the systemic rheumatic diseases are described here.

Laboratory findings in rheumatoid arthritis usually include a raised acute phase reactants like ESR and C-reactive protein levels, anemia, thrombocytosis, and leucocytosis. Rheumatoid factor is an autoantibody directed against the Fc region of IgG. Rheumatoid factor and IgG join to form immune complexes that contribute to the disease process. Various immunoassays are available for detection of rheumatoid factor. The classic Rose-Waaler test is hemagglutination test for rheumatoid factor in the serum, which depends on the ability of rheumatoid factor to agglutinate sheep erythrocytes coated with anti-sheep immunoglobulin.^[22] The latex agglutination test, in which latex particles coated with human IgG aggregate in the presence of IgM rheumatoid factor are also widely available. These tests identify only the IgM isotype of rheumatoid factor. ELISA can measure IgG, IgA, and IgM rheumatoid factors. It is important to mention here that some forms of rheumatoid arthritis like oligoarticular rheumatoid arthritis may be associated with a negative test for IgM rheumatoid factor but a positive test for IgG rheumatoid factor.^[23] However, the rheumatoid factor is a nonspecific marker, present in many normal individuals, patients with other systemic rheumatic diseases, and in chronic infections. It is found in approximately 70%–80% of patients of rheumatoid arthritis.^[24]

Wegener's granulomatosis (WG) or granulomatosis with polyangiitis (GPA) is a granulomatous vasculitis, typically involving small- to medium-sized vessels and characterized by formation of necrotizing granulomas within blood vessels. The antineutrophil cytoplasmic antibody (ANCA) test has been very helpful in the diagnosis of WG/GPA. ANCA, first described in 1982 by Davies *et al.*,^[25] are directed against antigens within the primary granules of neutrophils and monocytes. They are divided into cytoplasmic (c-ANCA) and perinuclear

(p-ANCA) subtypes based on their immunofluorescence patterns. The antigens responsible for these based on their immunofluorescence patterns and aetiopathogenesis for various autoimmune vasculitis have also been identified: proteinase 3 for c-ANCA and myeloperoxidase for p-ANCA. The sensitivity of cANCA is about 90% in active WG/GPA and specificity often exceeds 95%.^[26,27]

Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune disorder with heterogeneous presentation. SLE is characterized by autoantibodies including double-stranded DNA (dsDNA), anti-Smith, anti-Ro, and anti-La antibodies. The pathogenic significance of these antibodies is still unclear, but some like anti-dsDNA are very specific for SLE. Antinuclear antibodies (ANAs) are usually the first investigation commonly ordered in case of clinical suspicion of SLE and other various systemic rheumatic diseases. ANAs are usually detected by the immunofluorescence and their staining patterns, which is largely dependent on the location of the target antigen. When human cultured cells are used as the substrate, ANA can be considered as an ideal screening test because of its simplicity and sensitivity which is approximately 95%.^[28] However, some healthy individuals can test positive for this serological marker,^[29] and there are many conditions associated with a positive ANA, leading to the low specificity of ANAs for SLE.

Autoantibodies to single-stranded DNA (ssDNA) are nonspecific but antibodies to dsDNA are 95% specific for SLE and seen in 70% of SLE patients. Anti-Sm (Smith) antibodies are pathognomonic for SLE and found in approximately 30% of SLE patients. Anti-ribosomal antibodies are highly specific for the diagnosis of SLE, but they are less sensitive than anti-dsDNA or anti-Sm antibodies. Antinuclear ribonucleoprotein antibodies, anti-Ro (SS-A), and anti-La (SS-B) antibodies are not disease specific.^[22-24]

Diagnosis of Leprosy

Diagnosis of ocular inflammation due to leprosy primarily depends on detailed history taking, careful examination of eye, and proper and careful systemic examination. Characteristic skin lesions with sensory loss with or without thickened nerves and positive skin smears aid in diagnosis of this clinical entity. Difficulties in growing the bacilli *in vitro* are major limitation of the laboratory diagnosis of this clinical entity. Slit skin smears are collected with the edge of the scalpel blade turned perpendicularly and the material obtained is examined microscopically. Bacterial load is estimated through quantitative microscopy and the values are expressed in logarithmic scale, which is known as bacteriological index. Skin or nerve biopsy is required in cases with high degree of clinical suspicion with negative slit skin smears. Mitsuda or lepramin test, a delayed hypersensitivity test, is now days rarely used because of its limited sensitivity and specificity. PCR to detect the lepra DNA has been tried.^[30]

Diagnosis of Leptospirosis

Leptospirosis is a spirochetal disease of developing countries, especially in tropical areas which can multiple organs including eyes. Though intraocular inflammation due to leptospirosis is not very common, atypical and variable presentation, severity of the intraocular inflammation, underestimation of the clinical entity, and failure to start rapid treatment can lead to significant

visual morbidity. Diagnosis of leptospirosis usually depends on clinical suspicion, proper history, and positive serological test. Nowadays various serological tests are available for the diagnosis of leptospirosis and this includes micro-agglutination test, slide agglutination test, ELISA, PCR for leptospiral DNA, etc. Micro-agglutination test is the most sensitive and specific test for the diagnosis of systemic leptospirosis.^[31] Leptodipstick assay is a simple test for the detection of Leptospira-specific IgM antibodies in human blood sample. The test requires no special equipment, easy to perform, and rapid.

Diagnosis of Dengue and Chikungunya

Dengue virus infections are the most common mosquito-borne viral diseases of humans worldwide. The virus is endemic in 112 countries.^[32] Ocular involvement in dengue fever has been reported from various part of the world. According to a study by Chee *et al.*, reported prevalence of dengue maculopathy in hospitalized patients with dengue fever was 10%.^[33] Laboratory tests in such patients show a leucopenia and thrombocytopenia, with abnormalities of coagulation, liver function tests, and complement levels. The serological diagnosis of the disease is confirmed by positive IgM ELISA serology to the dengue virus which becomes detectable by about day 5 of the illness. Before this period, real-time polymerase chain reaction (RT-PCR) is helpful.

Diagnosis of ocular inflammation associated with chikungunya fever depends on elaborate history taking and positive serology tests. Principle of serological tests involves detection of anti-CHIC antibodies with IgM ELISA technique. However, the test becomes positive 4–5 days after the onset of fever. PCR, preferably RT-PCR techniques, are found to be more sensitive in laboratory diagnosis of chikungunya virus.^[34]

3. Radiological investigations

Radiological investigations are often very helpful in diagnosis of uveitis. Radiological evidence of bilateral hilar lymphadenopathy (BHL) is the most common radiological finding in systemic sarcoidosis and regarded as pathognomonic of this clinical entity. It is present in 50%–89% of cases of sarcoidosis and presence of BHL determines stage 1 of the pulmonary sarcoidosis.^[35] High-resolution computerized tomography (HRCT) has been found to be a useful boon in diagnosis of ocular TB. In a study by Ganesh *et al.*, HRCT chest findings in 80.9% of the patients with granulomatous uveitis were suggestive of healed or active TB.^[36] It has been seen that a computerized tomography (CT) scan is superior to conventional x-rays due to its ability to image the mediastinum, and may be useful in cases where a systemic focus of TB is strongly suspected.

The hallmark of the ankylosing spondylitis is the involvement of sacroiliac joints. X-ray imaging of sacroiliac joints in such patients may show loss of cortical margins, irregularities of the joint spaces, sclerosis of joint surfaces, and fusion of the joints in late stages. Ossification of the anterior longitudinal ligaments may lead to “bamboo spine.” CT and magnetic resonance imaging (MRI) can delineate the complex anatomy of the sacroiliac joints better than the conventional radiographs. MRI can detect sacroilitis before it is apparent on conventional radiography or CT because of its to detect bone marrow edema. Contrast-enhanced MRI studies increase further the sensitivity for detecting early sacroilitis.^[37]

4. Human leukocyte antigen (HLA) in uveitis

Human leukocyte antigen (HLA) is a protein present on the surface of the cells that constitutes the major histocompatibility complex (MHC). HLA genes are located in short arm of chromosome 6. Various HLA haplotypes are known to be associated with uveitic conditions.

HLA B51 (HLA B-5101) has a strong association with Behcet’s disease and can be considered in suspected patients.^[38,39] However, it should be kept in mind that presence or absence of the HLA-B51 does not confirms or excludes the possibility of the disease. It is worth mentioning here that this parameter is not included in the international classification criteria for the diagnosis of Behcet’s disease. In a study carried out in Japan, one of the countries with high incidence of Behcet’s disease showed that HLA-B51 is present in only 55% of diagnosed cases of Behcet’s disease.^[40]

HLA B 27 related uveitis is the most common cause of anterior uveitis world wide. HLA B 27 related uveitis includes ankylosing spondylitis, reactive arthritis (Reiter’s syndrome), psoriatic arthropathy, and inflammatory bowel syndrome. This group of inflammatory disorders represents a distinct clinical entity with clinically important ocular and systemic features.

It has to be kept in mind that though HLA typing is helpful in finding the association of the inflammation with particular HLA haplotype and to understand the pathophysiology of the many underlying disease processes or to detect previously undiagnosed systemic conditions, its role as diagnostic test of a particular inflammatory entity is limited and should be judiciously used.^[41]

5. Invasive diagnostic procedures

Though most of the inflammatory pathologies can be diagnosed by the clinical examination and available laboratory and ancillary investigations, sometimes few cases may present without characteristic clinical pictures or overlapping clinical features and the etiology remains obscure. In such cases invasive diagnostic procedures help us to reach an accurate diagnosis.

Anterior Chamber Paracentesis

Anterior chamber paracentesis has the advantage of being quick, relatively easy to perform, and can be carried out in the outpatient setup. In case of infectious uveitis and endophthalmitis, a portion of the aqueous aspirates should be sent to microbiology for direct smear for bacteria, fungus, acid fast bacilli, culture for bacteria, fungus, and mycobacteria, and PCR. Up to 0.2 mL of fluid can be obtained, which may be sometimes insufficient for the elaborate laboratory examination. Sometimes, posterior segment inflammation with relatively mild or nil anterior chamber inflammation can yield false-negative result with this procedure.

Vitreous Biopsy

Vitreous biopsy is indicated in cases of suspected intraocular infection, intraocular lymphoma, atypical intraocular inflammation not responding to conventional therapy, and conditions where larger sample is required. This can be

obtained by either a vitreous cutter or by using a 23 G needle. The procedure needs to be carried out by a skilled ophthalmic preferably a vitreoretinal surgeon. This allows up to 2 mL of undiluted vitreous to be collected for analysis. Material obtained from such procedures can undergo cytological evaluation, PCR, detection of intraocular antibodies (by ELISA), flow cytometry, and culture.

Biopsy of iris and Ciliary Body

Biopsy of iris and ciliary body is mainly performed in suspected tumors in these anatomical locations. However, in rare instances, this procedure is done to establish diagnosis in inflammatory granulomas.^[42]

Choroidal Biopsy

Choroidal biopsy is performed to investigate the choroiditis of unknown causes, choroidal malignancies, etc. The procedure can be performed via transscleral approach, ab interno/transvitreal approach, or by fine needle aspiration technique. However, the risks of choroidal hemorrhage and retinal detachment are the main limitations of this procedure. In some cases where retinal or choroidal tissues are desired, chorioretinal biopsy can be performed.^[43,44]

Retinal Biopsy

Retinal biopsy is carried out to diagnose the causes of unknown or atypical retinitis. Transvitreal route is used and often an undiluted vitreous specimen is obtained for vitreous biopsy.^[43,44] While performing retinotomy for obtaining the biopsy specimen, following points should be kept in mind:

- Junction of inflamed and normal retina should be given consideration, as periphery of an inflammatory area is more likely to harbor the infectious agents.
- Superonasal quadrant is preferred, because of less chances of damage to the macula from retinal detachment and easier tamponade postoperatively.
- Less vascular area and peripheral locations are preferred while selecting the site of biopsy for obvious reasons.

6. PCR: A new boon in laboratory diagnosis of uveitis

The 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 [Fig. 3]. 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.

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 can also be used to perform PCR. Tear fluid can be collected from eye wash by rinsing the ocular surface with 500 μ L of sterile saline.^[22] The choice of collecting sample should be guided by disease suspicion. 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.

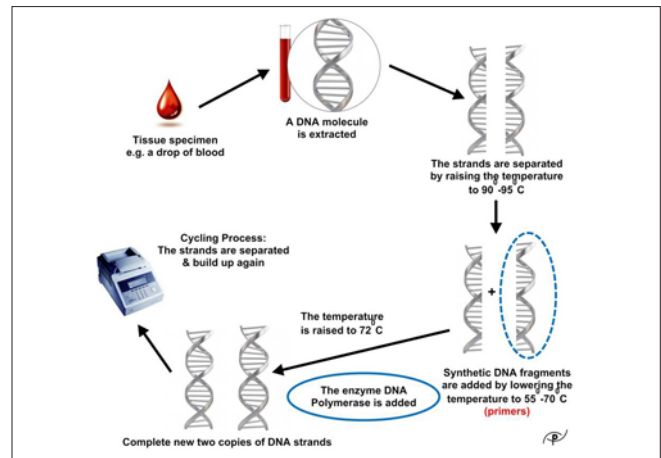


Figure 3: Principles of PCR

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/qPCR) or kinetic PCR. So, RT-PCR monitors the fluorescence emitted during the reaction at each PCR cycle in real time as opposed to endpoint detection.^[45-48]

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

Human herpes viruses can widely affect eye and ocular adnexa and their viral genome can be detected by PCR techniques. Removal of the drawback of endpoint PCR and ability to quantify the viral DNA have made the RT-PCR an ideal boon in the management of herpetic diseases. Hasegawa *et al.*^[49] with the help of RT-PCR technique analyzed 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 RT-PCR can be used to reliably diagnose herpetic keratitis and in cases with low copy numbers, diagnosis based on the RT-PCR is not recommended. PCR has been proven more than 90% sensitive for detection of VZV, HSV, and CMV.^[50-52] Knox *et al.*^[53] 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.

PCR has also been tried for detection of ocular toxoplasmosis. The use of intraocular antibody titre along with PCR yields higher sensitivity. Aouizerate *et al.*^[54] showed that the PCR combined with the determination of the Goldmann-Witmer coefficient 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.*^[55] were able to detect toxoplasma DNA in 80% cases

of suspected ocular toxoplasmosis. Mahalakshmi *et al.*^[56] showed a positive PCR result in 51.9% cases with clinically suspected ocular toxoplasmosis which was not significantly less than Goldmann-Witmer coefficient (72.7%). PCR along with restriction fragment length polymorphism analysis has been used to discover three antigenically identical strains of the *T. gondii*, which can be considered as an important milestone in the diagnosis and management of ocular toxoplasmosis.^[57] Recently, RT-PCR has been utilized as a rapid and sensitive technique for quantitatively evaluating ocular samples for the presence of *T. gondii*.^[58]

Although culture is considered as a “gold standard” in microbiological assessment of the diseases, endophthalmitis vitrectomy study has shown that 30% of cases of endophthalmitis were culture negative. PCR using 16 S ribosomal primer (all bacteria share common and highly repetitive DNA sequences for their 16S ribosomal RNA) yields faster result and was studied by Therese *et al.*^[59] 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.*^[60] analyzed 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 a great potentiality in establishing associations of pathogens to specific disease and it can be utilized to testify various hypotheses regarding infectious etiology of various diseases. Quentin and Reiber^[61] showed that patients with Fuch’s heterochromic cyclitis had raised intraocular antibody titre and positive RT PCR for rubella virus. Similarly, Chee *et al.*^[62] showed that 36% of their patients with either Posner-Schlossman syndrome or Fuch’s heterochromic iridocyclitis had positive CMV PCR.

Being a simple, rapid, sensitive, and specific technique, PCR has become a useful adjunct to the existing diagnostic procedure in the field of modern ophthalmology. With 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.

Tailored laboratory workup plays a crucial role in the diagnosis and management of uveitic patients. The differential diagnosis of uveitic conditions can be accomplished by a thorough and elaborate history taking, comprehensive eye examination and physical assessment. If diagnosed and treated on time, most of the uveitic conditions can be managed without long-term sequelae and complications.

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