Laboratory diagnosis of eye viral infections
Although treatment of viral infections is often non-specific, diagnosis assists the control of inappropriate treatment that could lead to more serious clinical sequelae, eg the application of steroids during infection with HSV allows the virus to multiply more rapidly.
Types of viruses causing eye infection

- **DNA viruses:**
  - Adenoviruses
  - Herpes viruses
  - Poxviruses (*Molluscum contagiosum*)
  - Papillomaviruses

- **RNA viruses:**
  - Picornaviruses
  - Togaviruses
  - Orthomyxoviruses and paramyxoviruses
  - Retroviruses
Family: Adenoviridae

viruses: different serotypes

Disease association:
- simple follicular conjunctivitis (multiple serotypes)
  - pharyngoconjunctival fever (most commonly serotype 3 or 7)
  - epidemic keratoconjunctivitis (EKC; usually serotype 8, 19, or 37, subgroup D)
- Laboratory diagnosis:
  - Virus isolation
  - Rapid immunodetection assays
  - Paired serologic titers 2-3 weeks apart
  - DNA based techniques
- Specimens:
  - Conjunctival swabs
Family: Herpesviridae
Viruses: HSV-1 & 2

- **Disease association:**
  - blepharoconjunctivitis
  - epithelial keratitis
  - stromal keratitis
  - iridocyclitis
- **Laboratory diagnosis:**
  - viral culture
  - antigen- or DNA-detection methodologies.
  - Serologic tests for neutralizing or complement-fixing (for primary infection)
- **Specimens:**
  - Vesicles can be opened with a needle, and vesicular fluid cultured.
  - Scrapings from the vesicle base can be tested by cytology or for the presence of HSV antigen, Conjunctival scrapings or impression cytology specimens can be similarly analyzed.
- **Family**: Herpesviridae  
- **Viruse**: Varicella Zoster

- **Disease association:** dermatoblepharitis, conjunctivitis, Keratitis  
- **Laboratory diagnosis:** immunodiagnostic methods, viral culture, PCR  
- **Specimens:** As with HSV, scrapings from a vesicle base can be tested by cytology, PCR, or culture, or for the presence of VZV antigen. Conjunctival scrapings or corneal impression cytology specimens can be similarly analyzed.
Family: Herpes viridae

Viruse: Epstein-Barr

- **Disease association:**
  - dacryoadenitis,
  - conjunctivitis,
  - keratitis
- **Laboratory diagnosis:**
  - Because of difficulty in viral isolation, diagnosis of EBV infection depends on the detection of antibodies to various viral components. During acute infection, first IgM and then IgG antibodies to viral capsid antigens (VCA) appear. Anti-VCA IgG may persist for the life of the patient. Antibodies to early antigens (EA) also rise during the acute phases of the disease and subsequently decrease to low or undetectable levels in most individuals. Antibodies to EBV nuclear antigens (EBNA) appear weeks to months later, providing serologic evidence of past infection.
- **Specimens:** Serum and eye fluid samples
- **Disease association:**
  - Retinitis

- **Laboratory diagnosis:**
  - Virus isolation
  - PCR
  - Ag detection, CMV retinitis can be identified by an ophthalmologist, who examines internal eye structures to check for characteristic abnormalities using an ophthalmoscope.
  - Vitreous sampling for culture or PCR is sometimes useful, but this procedure is not without risk
Family : Poxviridae  
Viruse: Molluscum contagiosum

- **Disease association:**  
  Infection produces 1 or more umbilicated nodules on the skin and eyelid margin and, less commonly, on the conjunctiva. Eyelid nodules release viral particles into the tear film.

- **Laboratory diagnosis:**  
  Molluscum contagiosum virus cannot be cultured using standard techniques. Diagnosis is based on the detection of the characteristic eyelid lesions in the presence of a follicular conjunctivitis. No testing available in routine clinical laboratories.
Family: Picornaviridae
Viruses: Enterovirus type 70 and coxsackievirus A24

- **Disease association:**
  - Acute hemorrhagic conjunctivitis (AHC)
- **Laboratory diagnosis:**
  - Virus isolation
  - PCR
  - Specimens:
  - Conjunctival Swab
Sampling for eye infections

- SPECIMEN COLLECTION
- SPECIMEN TRANSPORT AND STORAGE
- SPECIMEN PROCESSING
Sampling

external

internal
• External specimens should be placed into Virus Transport Medium (VTM) immediately after collection.
• Samples collected after the application of fluorescent dye to the patient’s eye do not appear to affect the isolation of virus by cell culture.
- TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING
- Specimens that may be delayed should be refrigerated prior to transportation to the laboratory
- Serum, aqueous and vitreous samples that may delayed for a long time for examination or nucleic acid extraction should be stored at -20°C
• Viruses associated with infections of the eye are in Hazard Group 2;
• Laboratory procedures that may give rise to infectious aerosols, eg vortexing swabs, must be conducted in a microbiological safety cabinet and the operator should wear gloves. Chance contact of infected gloved hand with the operator’s eye must be avoided as laboratory acquired infection would be a likely outcome.
Conventional virus culture and examination of cytopathic effect may be used both for adenoviruses and HSV. However an alternative method for adenovirus detection is the use of a shell vial culture system although it may be less sensitive than conventional culture. Detection of HSV and adenovirus from eye material using direct immunofluorescence or EIA techniques are sub optimal. These viruses usually require amplification in culture prior to performing these techniques. Molecular methods of detection are also available.
The laboratory diagnosis of ocular adenovirus infection is a function of the onset of clinical symptoms. The earlier the conjunctival samples are collected after clinical onset, the higher likelihood of a positive result. The adenoviral load of viable virus and antigen decreases over time. Four tests can be used for laboratory diagnosis: 1) cell culture, 2) shell vial (no longer used routinely), 3) EIA (like Adenoclonetm) (no longer used routinely) or direct immunofluorescence. These viruses usually require amplification in culture prior to performing these techniques, and 4) PCR. PCR may possibly be more diagnostic in specimens collected at the late onset of infection and for non-culturable Adenovirus present in some cases.
Specimens are directly collected by vigorously swiping the exposed conjunctiva with a plastic soft-tipped applicator. Cornea samples are not necessary. Topical anesthetic can be applied to the conjunctiva but this is optional. Collected samples are placed in 2.0 ml of viral transport medium. Viral culturettes can also be used for transportation to the laboratory and these can be transferred to the viral medium. All laboratory testing can be processed from the 2.0 ml of transport medium. Adenovirus is not a fastidious virus. It will remain viable under many conditions and collected samples should be easily transported through mail carriers.

**Specimen processing**

- The swab should be agitated to release maximum material into the virus transport medium. This should be carried out within a microbiological safety cabinet.

**Specimen Collection (adenovirus)**
Cell culture
The "gold" standard for adenovirus laboratory testing is cell culture. Collected samples are layered on a monolayer of cells in test tubes. If present, Adenovirus will present as cytopathic effect of rounded cells. The cytopathic effect of Adenovirus is confirmed for the presence of antigen by EIA (Adenoclone™). The best cell-line for testing Adenovirus is A549. This is a human carcinoma continuous cell-line. When samples are collected within one to three days of clinical onset, cell culture generally is positive within four to seven days. Samples collected after three days may take one to three weeks to produce cytopathic effect. Cell culture will confirm an adenovirus diagnosis but it may not provide timely results for immediate patient care. All virology laboratories can offer cell culture isolation for Adenovirus.
Shell Vial Culture
Shell vial is another cell culture test but the results are ready in three days. Vials of A549 cells are inoculated with collected samples and centrifuged. The vials are then incubated and stained at day three with immunofluorescent antibodies specific to Adenovirus. The cells infected with Adenovirus will light up with examination under a fluorescent microscope. Shell vial highly correlates with standard cell culture especially when samples are collected within seven days of clinical onset. Shell vial testing is processed by many virology laboratories, especially those involved with respiratory virus. Shell vial testing is highly recommend for diagnosing ocular adenovirus infection.
EIA (Adenoclonetm)
Adenoclonetm is an enzyme immunoassay that can detect adenoviral antigen from collected ocular specimens. Positive results can be obtained within 75 minutes. Unfortunately, Adenoclonetm is only 40 to 50 percent sensitive in detecting adenoviral antigen from clinical specimens. A high load of antigen is necessary for a positive test and this correlates with collection within one to three days of clinical onset. The power of this test is that it does provide rapid results when tests are positive but negative tests need to be confirmed with cell culture or shell vial. Adenoclonetm is not widely offered by diagnostic laboratories.
Adenovirus

PCR
Polymerase Chain Reaction (PCR) is a molecular test that amplifies specific adenoviral DNA sequences from clinical samples and then identifies the amplified products with gel techniques. PCR is a highly sensitive and specific test that can detect adenoviral DNA from clinical samples. Results can now be obtained within one to three days.
- Advances in the diagnosis of viral diseases of the eye included
- (1) the use of the phase microscope for the rapid recognition of inclusion bodies,
- (2) improved staining of elementary bodies,
- (3) recognition of a mononuclear exúdate as a characteristic of viral infection of the conjunctiva and cornea,
- (4) introduction of a skin test for herpes simplex virus infections
The detection of Herpes Simplex (HSV) from ocular specimens is essential for prompt and accurate therapy. HSV can be detected from ocular samples with cell culture, ELVIS™ (Enzyme Linked Viral Induced System) and PCR.

In advanced laboratories, all clinical ocular samples suspected of possible HSV infection are tested with standard cell culture, ELVIS™, and PCR. The processing all specimens with this battery of tests provides the most expedited results especially when the results can be delayed due to off-hour collection, weekends, and holidays.
Cell Culture
The "gold" standard for HSV laboratory testing is cell culture. Collected samples are layered on a monolayer of cells in test tubes. If present, HSV will present as cytopathic effect of rounded cells. A549 cells as the cell-line for testing HSV. This is a human carcinoma continuous cell-line. Some other cell lines can be used for virus isolation. When samples are positive for HSV, cytopathic effect is generally present within one to three days. It is rare, but sometimes one to two weeks is necessary to isolate HSV in cell culture. Cell culture will confirm an HSV diagnosis but it may not provide timely results for immediate patient care. All virology laboratories can offer cell culture isolation for HSV.
ELVIS™

ELVIS™ (Enzyme Linked Viral Induced System) is another cell culture test but the results are ready in ONE day. Cells infected with HSV commence a cascade of reactions that results in the accumulation of beta-galactosidase in the cells. Viral samples are layered on the specially engineered cell line in a shell vial and centrifuged. The vial is incubated for one day (overnight), fixed, and stained with a substrate that reacts with the beta-galactosidase. The reactions result in a blue-color change in the cells that are observed with an inverted microscope. ELVIS™ testing may also be limited in its availability. ELVIS™ testing is highly recommend for diagnosing ocular HSV infection.
PCR
PCR is performed for HSV (1 or 2) on specimens collected by soft-tipped applicators, metal spatulas, and jeweler's forceps, and placed in 2.0ml of chlamydia transport medium. Intraocular fluid or vitrectomy specimen can be supplied directly or increased to a volume of 0.45 ml with chlamydia transport medium. 0.45 ml of the medium is supplied for PCR testing
Which test and which sample are the best?
Comparison of different tests and samples for virus detection (HSV-1)

**Results** HSV 1 antigen was detected in 31/229 (13.53%) tear specimen and 35/153 (22.87%) corneal scrapings in immunofluorescence assay; virus was isolated from 12/229 (5.2%) tear and 17/153 (11.11%) corneal scrapings, and PCR was positive for both the genes in 32/229 (13.97%) tear specimen and 56/153 (36.66%) corneal scrapings.

**Conclusion** Corneal scrapings yielded a significantly better HSV positivity than tears in both the PCR assay (p<0.0005) and immunofluorescence assay. PCR was much more sensitive than immunofluorescence and virus isolation. However, tears should be tested for definitive laboratory diagnosis of HSV infection whenever corneal scraping collection is not possible.
A 13-Year Retrospective Review of Polymerase Chain Reaction Testing for Infectious Agents from Ocular Samples

**Results**
Polymerase chain reaction results were positive more often than culture results for HSV ($P = 0.0001$), VZV ($P = 0.00001$), *C. trachomatis* ($P = 0.00005$), and *Acanthamoeba* ($P = 0.04$). For adenovirus, cell culture isolation results were positive more often than PCR results ($P = 0.001$). Polymerase chain reaction was the primary diagnostic test for detecting cytomegalovirus and *Toxoplasma*.

**Conclusions**
The current study demonstrated the importance of PCR as a routine diagnostic test for detecting both common and infrequent ocular pathogens. Cell culture isolation is still a definitive test for adenovirus and a confirmatory test for HSV and *Acanthamoeba*.

**Methods**
The daily laboratory logs for diagnostic testing were reviewed for PCR, cell culture isolation, shell vial isolation, and *Acanthamoeba* isolation from January 1997 through May 2010 for herpes simplex virus (HSV), adenovirus, varicella zoster virus (VZV), *Chlamydia trachomatis*, *Acanthamoeba*, and infrequent pathogens of intraocular inflammation.
Direct immunofluorescence assay (IFA) for HSV-1 antigen, polymerase chain reaction (PCR) for HSV-1DNA, and viral isolation on Vero cell line culture.

Positive samples by cell culture were 20.8%, whereas PCR was positive in 29.2%, and IFA was positive in 33.3%. IFA had better sensitivity (80%) and negative predictive value (81.8%) than PCR (70% and 76.9%, respectively); however, PCR had better specificity (71.4%) and positive predictive value (63.6%). This indicates that a combination of cell culture, IFA and PCR constitutes the best set of tools for diagnosis of clinically suspected cases of HSK.

Documented infection can be further assessed by cell-culture technique or PCR depending laboratory availability.
Fig. 1. Patients positive for HSV-1 infection by PCR, IFA, and viral isolation test.
• Correlation between clinical diagnosis and PCR analysis of serum, aqueous, and vitreous samples in patients with inflammatory eye disease

• PCR exam in the vitreous was more helpful as an auxiliary diagnostic test in the cases of CMV and ARN than PCR test of serum and aqueous.

• The time of the onset of the disease in this study did not appear to influence the results of the PCR analysis.

• Quality control of collection and analysis samples for PCR analysis should be high in order to avoid contamination and false positive results.

• PCR exam is a auxiliary diagnostic procedure that should be evaluated with ophthalmological aspects of the patient.

• Antibody detection in tear comparing to interocular fluids

• Further studies are needed to compare the tear and intraocular levels of CMV-specific antibodies in patients with retinitis to find out if CMV antibody testing in tear fluid could substitute for more invasive diagnostic procedures.
Infectious Uveitis

PCR (Real time PCR)

Goldmann-Witmer Coefficient (GWC)

GWC = (specific anti-virus IgG in aqueous humor/total IgG in aqueous humor)/(specific anti-virus IgG in serum/total IgG in serum).

A value of 2 or 3 is considered evidence of intraocular Ab synthesis.
Relationship between positive diagnosis and time of sampling after onset of viral and parasitic ocular disease. Bars show percentages of polymerase chain reaction (PCR; black bars), Goldmann-Witmer coefficient (GWC; white bars), and both PCR and GWC (hatched bars) positive samples/total number of positive samples.
Combining the techniques significantly improves the diagnostic sensitivity. Therefore, at least both PCR and GWC determination might be performed for comprehensive diagnosis of intraocular infections.