Perspective

Diagnosis of viral and chlamydial keratoconjunctivitis: which laboratory test?

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Table 1  Viral and chlamydial causes of infectious conjunctivitis

<table>
<thead>
<tr>
<th>Causative agent (references)</th>
<th>Differential diagnosis</th>
<th>Epidemiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA viruses:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenovirus (3)</td>
<td>EKC</td>
<td>Primarily types 8 and 19, but types 2–5, 7, 9, 11, 14, 16, 21–23, and 37 have also been associated with EKC. Highly contagious and transmitted by hand-to-eye contact, instruments, and solutions.</td>
</tr>
<tr>
<td>HSV (4, 5)</td>
<td>PCF</td>
<td>Primarily types 3, 4, and 7; occasionally type 5. Contagious; droplet transmission, particularly in families.</td>
</tr>
<tr>
<td>RNA viruses (3):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Picornavirus</td>
<td>NSFC</td>
<td>A number of serotypes have been reported including 1–11, 14–17, 19, 20, 22, and 26.</td>
</tr>
<tr>
<td>Measles virus (6)</td>
<td>Conjunctivitis; keratitis or KC</td>
<td>HSV-1 or HSV-2; conjunctivitis is most common in adults and children 1–5 years of age; recurrent disease is common; leading infectious cause of blindness in developed countries.</td>
</tr>
<tr>
<td>Chlamydia:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. trachomatis (7)</td>
<td>AHC</td>
<td>EV70; CA24; common (epidemic or endemic forms) in developing countries; highly contagious. Common cause of childhood blindness in developing countries. HSK may complicate measles in developing countries.</td>
</tr>
<tr>
<td></td>
<td>MVO</td>
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</table>

The need for laboratory investigation

Owing to the limited reliability of clinical diagnosis of adenovirus, HSV, and C. trachomatis induced keratoconjunctivitis, accurate laboratory investigation for these agents in conjunctival swabs is often valuable. Failure to diagnose ocular adenoviral disease can result in outbreaks of epidemic keratoconjunctivitis. Prompt recognition of the strains of adenovirus causing this condition in patients can, however, help to contain the development of a hospital-based epidemic. C. trachomatis infection (particularly with serovars D–K) is usually associated with asymptomatic genitalurnary colonisation. Misdiagnosis of this ocular infection may represent a missed opportunity to detect infection of the genital tract which can ultimately result in a series of complications including pelvic inflammatory disease, epididymitis, ectopic pregnancy, and infertility in the patient and/or partner.

Conventional laboratory techniques

The conventional techniques for diagnosing viral and chlamydial keratoconjunctivitis include conjunctival cytological investigation; inoculation of susceptible cell lines followed by observation of cytopathic effect or visualisation.
Compared with DIF.
†Compared with clinical diagnosis.
‡Compared with PCR.
IC=immunochromatography; IDB=immune dot blot.
DIF=direct immunofluorescence; IPO=immunoperoxidase; EIA=enzyme

using various chemical or immunological staining
techniques; examination of blood and/or tears for vari-
sious classes of antibodies; and detection of viral or
chlamydial antigens in conjunctival and corneal
specimens. However, the pitfalls of these
techniques are numerous. Traditional cyto-
logical investigation is insensitive and subjective.
Cell culture isolation requires viable organisms necessitating
special transport media and prompt transport of spec-
imens between patient and laboratory. It is costly and time
consuming but remains the “gold standard” as isolation of
an infectious agent is definitive and allows further charac-
terisation.

Serodiagnosis (IgM antibody detection) of infection is not always possible during the acute stages of illness and false positive results may arise because of cross reactivity with other related pathogens, or if the tear film is assessed, through the transudation of antibodies from blood into

tears. Antigen detection techniques circumvent the re-
quirement for preservation of infectivity required for
culture but can produce false negative and false positive

test results. In addition, the wide range of sensitivity and
specificity values obtained with antigen detection tests
(Table 2) is not only influenced by the quality of the test
itself, but by the quality of the standard test used (cultur-
e or other comparative test); the “cut off” value for
positive and negative results; the experience of the observer
to discriminate between specific and non-specific staining
in the case of the direct immunofluorescence test; and false
positive results due to cross reactivity with other pathogens
in the case of immune immunoassays.

The introduction of diagnostic test procedures based on
nucleic acid amplification techniques has led to a
reappraisal of the use of conventional methods of
diagnosis. Nucleic acid amplification techniques currently
available or under active development include the
polymerase chain reaction (PCR), the ligase chain reaction
(LCR), transcription based amplification system, Qβ repli-
case amplification, cycling probe reaction, strand displace-
ment amplification, and branched DNA signal
amplification. Of these techniques, PCR, the first of the
nucleic acid amplification methods to be described, is the
most developed.

Polymerase chain reaction (PCR)
PCR allows the identification of extremely small quantities of
DNA with a very high degree of specificity. The test has
revolutionised the diagnosis of microbial diseases and
since its first use in eye disease in 1990, the test is now
being widely used in clinical ophthalmology. Owing to
the sensitivity and speed of the PCR, and its use of small
sample volumes, the technique has been shown to be of
great value in the diagnosis of infections involving both the
ocular surface and the orbit (Table 3). A number of
studies have evaluated the use of PCR as a diagnostic tool
for the detection of adenovirus, HSV, and C. trachomatis
in conjunctival swabs.

ADENOVIRUS
PCR may be the only means by which rapid and reliable
diagnosis of adenoviral keratoconjunctivitis can be
achieved. The technique has been shown to be more sensi-
tive, accurate, and rapid than culture for detecting and
typing adenovirus in cases of conjunctivitis. In addition,
PCR appears to be more sensitive than immunoassay in
detection of ocular adenovirus infections. For example,
Kinchington et al evaluated the application of PCR in 107
corneal swab samples and correlated the results with
those obtained with tissue culture and a commercial
immunoassay (Adenoclone). The PCR was positive in 46
of 58 adenoclone negative, culture positive swabs, and in
11 of 11 adenoclone positive, culture positive swabs. Only
one of 38 non-adenoviral ocular swab samples was positive
by PCR giving an overall specificity of 97.3%. These
results demonstrate the superiority of PCR in terms of
speed over tissue culture isolation and in sensitivity over
immunoassay.

In our own laboratory, the overall sensitivity of PCR for
detection of adenovirus in eye swabs was similar to that of
cell culture and superior to that of antigen detection by
immuno dot-blot test. More recently, using a newly
designed adenovirus primer pair, we have a PCR which is
significantly more sensitive than culture. Of 415 eye swabs
positive by culture and/or PCR, 386 (93%) were positive
by PCR compared with 248 (59%) by cell culture.

HERPES SIMPLEX VIRUS
Few studies have compared PCR with conventional
techniques. This is probably because the clinical features of
herpetic eye disease are often strongly suggestive and cul-
ture results are available much sooner (3 days) than those
of adenovirus (up to 4 weeks) and chlamydia (5 days).
Nevertheless, owing to its speed and sensitivity, PCR is a
useful diagnostic tool for HSV infection of the ocular
surface. Yamamoto et al utilised PCR for the
detection of HSV genomic sequences in tear film from
patients with clinically diagnosed HSV keratitis. The PCR
was positive in 12 of 12 epithelial keratitis specimens, two
of six stromal keratitis, but in none of 20 normal
specimens. In another study by Kowalski et al utilising
cell culture as a gold standard test, PCR was shown to be
significantly more sensitive (95%) in diagnosing herpetic ocular disease than diagnosis by clinical examination (55%).

**CHLAMYDIA TRACHOMATIS**

For *C. trachomatis*, the PCR has been shown to be as or more sensitive than conventional techniques.57 86–95 Bobo *et al* investigated the use of PCR in 234 Tanzanian children who were clinically classified as having no sign of trachoma (*n*=97), follicular trachoma (*n*=100), or intense inflammatory trachoma (*n*=37). The PCR detected *C. trachomatis* in 24%, 54%, and 95% of subjects, respectively, compared with detection by direct immunofluorescence of 1%, 28%, and 60%, respectively. The overall prevalence of chlamydial eye infection was 48% by PCR compared with 22% by direct immunofluorescence. In addition, this study showed the utility of PCR as a suitable method for field use owing to ease of specimen collection and transport.

In the United States, Talley *et al* screened ocular swabs from 30 consecutive patients with follicular conjunctivitis for adenovirus, HSV, and *C. trachomatis*. In this study, PCR was compared with cell culture and direct immunofluorescence for the detection of *C. trachomatis*. Only four patients were positive for *C. trachomatis* by PCR. Two of the four patients were positive by cell culture and one of four patients was positive by direct immunofluorescence. Thus, PCR appeared to be an equally specific and more sensitive method than cell culture or the direct immunofluorescence test. In another study Tabrizi *et al* compared culture and PCR techniques in the detection of *C. trachomatis* in 200 clinical samples including eye swabs (30%). A total of 173 specimens were assessable by PCR of which 24 (13.8%) were positive by both methods and four specimens were positive by PCR and negative by culture. Overall the PCR was 100% sensitive and 97.3% specific compared with cell culture. Nine and discharge of the patient from clinic (where cases of adenoviral keratoconjunctivitis pose a real threat to other patients and staff) test results would be required within 10–30 minutes. At the present time only immunochromatography can provide information within such a time frame but the low sensitivity of such tests is a major drawback.

**Table 3** Applications of PCR in ophthalmic infectious diseases

<table>
<thead>
<tr>
<th>Causative agent</th>
<th>Ocular site</th>
<th>Clinical diagnosis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Viruses:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Conjunctiva</td>
<td>KC</td>
<td>67–72</td>
</tr>
<tr>
<td>HSV</td>
<td>Tear film</td>
<td>keratitis</td>
<td>73, 74</td>
</tr>
<tr>
<td>EV70</td>
<td>Conjunctiva</td>
<td>keratitis or KC</td>
<td>82, 21, 75, 76</td>
</tr>
<tr>
<td>CMV</td>
<td>Aqueous</td>
<td>retinal necrosis</td>
<td>78, 78, 77, 78</td>
</tr>
<tr>
<td>HPV</td>
<td>Conjunctiva</td>
<td>uveitis</td>
<td>80</td>
</tr>
<tr>
<td>EBV</td>
<td>Aqueous</td>
<td>retinal necrosis</td>
<td>81</td>
</tr>
<tr>
<td>EV70</td>
<td>Aqueous</td>
<td>ophthalmic zoster</td>
<td>82</td>
</tr>
<tr>
<td>CMV</td>
<td>Aqueous</td>
<td>chorioretinal inflammatory disease or retinitis</td>
<td>83, 84, 83</td>
</tr>
</tbody>
</table>
| HPV             | Conjunctiva | conjunctival carci
| **Bacteria:**   |             | noma             | 85        |
| *C. trachomatis*| Conjunctiva | chlamydial conjunctivitis | 56, 86–95 |
| *Mycobacteria*  | Vitreous    | endophthalmitis   | 96        |
| *Parasites:*    |             |                   |           |
| *Toxoplasmagondii* | Aqueous and vitreous | retinochoroiditis | 78 |
| *Ocular tissue sections* | | ocular toxoplasmosis | 97 |

KC=keratoconjunctivitis; EV70=enterovirus 70; AHC=acute haemorrhagic conjunctivitis; VZV=varicella zoster virus; EBV=Epstein-Barr virus; CMV=cytomegalovirus; HPV=human papillomavirus.

Because of its sensitivity, false positive results due to the carryover of small amounts of the products of previous PCR tests may be encountered. Such problems can be avoided by strict application of a variety of preventative measures.94 False negative results may arise as a result of failure of PCR amplification. The most common cause of PCR failure is due to the introduction of inhibitors with the test sample.95 A variety of sample preparation techniques are now available to help avoid such problems and control for sample inhibition can be accomplished by the inclusion of internal control molecules96 or by amplification of human DNA in the sample (for example, β-globin gene).97

**AUTOMATION**

Nucleic acid amplification procedures may be automated to improve the reproducibility, speed, and overall cost of tests. An example is the Cobas Amplicor (Roche Molecular Systems) automated PCR system101 and the ligase chain reaction test kit (Abbott Diagnostics Ltd).102 While commercial test procedures and automation are, in relation to ocular disease, currently only available for *C. trachomatis*,103, 104 the available test repertoire is increasing. The more widespread application of these tests is anticipated, particularly as they overcome the problem of variable sensitivity and specificity between laboratories using their own “in house” procedures.

**Discussion and conclusions**

The ideal test for diagnosing ocular pathogens has been defined as one whose result is available before the patient leaves the doctor’s office.105 However, achieving sensitivity, specificity, low cost, long term shelf storage, and ease of performance by office staff without the need for expensive equipment or hazardous reagents currently presents a formidable technological challenge. Although PCR achieves the accuracy needed for diagnosis of ocular infectious agents, it still lacks the speed and simplicity required for an office based test. The anticipated introduction of effective treatment of adenoviral keratoconjunctivitis107 poses a real challenge to the applicability of the emerging tests discussed in this review. To allow dispensing of treatment and discharge of the patient from clinic (where cases of adenoviral keratoconjunctivitis pose a real threat to other patients and staff) test results would be required within 10–30 minutes. At the present time only immunochromatography can provide information within such a time frame but the low sensitivity of such tests is a major drawback.
The choice of the optimal technique for diagnosing viral and chlamydial keratoconjunctivitis depends upon the efficiency, speed, and cost of the test. The performance of conventional laboratory procedures is relatively poor and the interpretative difficulties documented with these tests are well recognised. Owing to their inherent sensitivity and high specificity, nucleic acid amplification procedures, in particular PCR, are recognised as the ultimate modern diagnostic tool for the identification of adenovirus, HSV, and C trachomatis in clinical eye swab samples. However, in laboratories without access to PCR, the conventional techniques of enzyme immunoassay, culture, and direct immunofluorescence are useful for diagnosing adenovirus, HSV, and chlamydia. The direct immunofluorescence test can, however, only be recommended if a small number of specimens are submitted and examined by skilled and experienced microscopists and although commercial enzyme immunoassays have the advantages of low cost and suitability for testing large numbers of specimens, the false positive and false negative results generated are serious disadvantages. Particularly in relation to C trachomatis infection, inaccurate diagnosis in a patient being investigated for conjunctivitis and not sexually transmitted disease, can lead to difficult communication with the patient and even litigation.

A conceptual difficulty with all nucleic acid amplification procedures is the finding of positive detection of nucleic acid in the absence of viable organisms (as determined by culture) or of antigen. The current sophistication of PCR means that provided due attention is given to intralaboratory quality control including rigorous application of anti-cross contamination procedures, the finding of the nucleic acid of an infectious organism in an ocular specimen is significant. The presence of residual nucleic acid usually indicates that infection persists well beyond the period indicated by cell culture or antigen detection tests. Factors generated in the course of the clinical response such as the elaboration of antibodies, interleukin 1, interferon gamma, and tumour necrosis factor (TNF) might inhibit or alter the metabolic capacity, surface receptor expression, or infectivity and therefore culturability or the antigenicity of the organism. In this way the infectivity might fall while the organism, containing DNA, is still detectable by PCR.

Nucleic acid amplification may, as has happened with other non-culture diagnostic procedures, also detect an infectious agent in circumstances which are not consistent with the clinical features under investigation. Such findings should not be dismissed because these diagnostic procedures have proved to be a powerful tool in elucidating the aetiology of several ocular diseases previously classified as idiopathic.

A further item of value in detection of nucleic acid in ocular specimens is in the assessment of treatment failure. Until recently, treatment failure was defined solely on the basis of positive culture. For the reasons outlined above, the lack of detection of infectious organisms may not signal the end of a disease process. Non-culture test results, particularly those of high sensitivity, are very probably a more reliable indicator of treatment failure. The continued development of nucleic acid amplification procedures will also lead to a reduction in the overall cost of diagnosing viral and chlamydial keratoconjunctivitis. Already PCR procedures are available to allow the simultaneous investigation of several pathogens using one test in which the three or more organism specific oligonucleotide primer pairs are included (multiplex PCR). Such tests allow the testing of a single sample and permit easy identification of possible, albeit rare, concurrent infection. In our own laboratory, for example, a multiplex PCR has been developed for the simultaneous investigation of adenovirus, HSV, and β globin in ocular swabs and, currently, we are modifying the technique to include C trachomatis detection.

In conclusion, nucleic acid amplification procedures are valuable modern techniques for the diagnosis of infectious ocular disease. The availability of automation and the more widespread availability of commercial assays will facilitate the use of these techniques in routine laboratories. These techniques are evolving rapidly and are likely to have an increasingly important role in therapeutic management.

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