

## PERSPECTIVE

# Diagnosis of viral and chlamydial keratoconjunctivitis: which laboratory test?

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Conjunctivitis and keratitis are common forms of ocular morbidity seen in general practice and eye units.<sup>1 2</sup> The aetiology of these diseases includes viral, bacterial, or parasitic infection as well as allergy, trauma, and dietary deficiency. Among the common microbial causes<sup>3-7</sup> (Table 1) are adenovirus, herpes simplex virus (HSV), and *Chlamydia trachomatis*. Ocular adenovirus infections occur throughout the world in both sporadic and epidemic forms, and large scale outbreaks of epidemic keratoconjunctivitis can occur in hospitals, schools, military establishments, or factories.<sup>8</sup> HSV type 1 ocular infection occurs in all countries with an annual incidence of up to 20.7 per 100 000 population and is the most common infective cause of blindness in developed countries.<sup>4 9</sup> Trachoma caused by *C trachomatis* serovars A-C is the leading infectious cause of blindness in the world and is a major public health problem in developing countries.<sup>10</sup> Adult chlamydial conjunctivitis, caused by *C trachomatis* serovars D-K, is an oculogenital infection and up to 90% of patients have concurrent genital infection.<sup>11-13</sup> Chlamydial neonatal conjunctivitis (ophthalmia neonatorum) develops in 18%-74% of babies born to mothers with genital chlamydial infection.<sup>7</sup>

This article reviews available diagnostic laboratory techniques for keratoconjunctivitis caused by adenovirus, HSV, and *C trachomatis* with special emphasis on modern molecular diagnostic techniques. For information on the clinical features, epidemiology, and treatment of these infections the reader is referred to a number of other reviews.<sup>8 9 14-17</sup>

## The need for laboratory investigation

Owing to the limited reliability of clinical diagnosis of adenovirus, HSV, and *C trachomatis* induced keratoconjunctivitis,<sup>18-23</sup> 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.<sup>24</sup> *C trachomatis* infection (particularly with serovars D-K) is usually associated with asymptomatic genitourinary colonisation.<sup>25</sup> 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.<sup>11-13 25</sup> In addition, delay in diagnosing chlamydial conjunctivitis and the use of inappropriate antibiotic treatment might trigger chlamydial persistence.<sup>26</sup> The availability of appropriate treatment for chlamydia,<sup>16</sup> HSV,<sup>17</sup> and possibly adenovirus<sup>27</sup> together with the potentially serious residual morbidity of these infections clearly justifies the need for accurate laboratory investigation in cases of keratoconjunctivitis.

## Conventional laboratory techniques

The conventional techniques for diagnosing viral and chlamydial keratoconjunctivitis include conjunctival cytological investigation<sup>28-30</sup>; inoculation of susceptible cell lines followed by observation of cytopathic effect or visualisation

Table 1 Viral and chlamydial causes of infectious conjunctivitis

Causative agent (references)	Differential diagnosis	Epidemiology
<b>DNA viruses:</b>		
Adenovirus (3)	EKC	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. Primarily types 3, 4, and 7; occasionally type 5. Contagious; droplet transmission, particularly in families.
	PCF	
HSV (4, 5)	NSFC	A number of serotypes have been reported including 1-11, 14-17, 19, 20, 22, and 26.
	Conjunctivitis; keratitis or KC	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.
<b>RNA viruses (3):</b>		
Picornavirus	AHC	EV70; CA24; common (epidemic or endemic forms) in developing countries; highly contagious.
Measles virus (6)	MVO	Common cause of childhood blindness in developing countries. HSK may complicate measles in developing countries.
<b>Chlamydia:</b>		
<i>C trachomatis</i> (7)	NCO	The most common form of infectious ophthalmia neonatorum today; occurs in babies born to mothers with genital infection serovars D-K.
	ACO	Oculogenital infection caused by serovars D-K, sometimes by B, and rarely by C; common in developed countries and urban areas of developing countries.
	Trachoma	Most common cause of preventable blindness in the world and common in rural areas of developing countries particularly in Africa, the Middle East, and Asia; caused by serovars A-C.
	LGV conjunctivitis	Very rare and is caused by serotypes L-1, L-2, and L3; transmission either venereally or accidentally in the laboratory.

EKC=epidemic keratoconjunctivitis; PCF=pharyngoconjunctival fever; NSFC=non-specific follicular conjunctivitis; KC=keratoconjunctivitis; AHC=acute haemorrhagic conjunctivitis; MVO=measles virus ophthalmia; NCO=neonatal chlamydial ophthalmia; ACO=adult chlamydial ophthalmia; LGV=lymphogranuloma venereum; HSK=herpes simplex keratitis; HSV=herpes simplex virus; EV=enterovirus; CA=Coxsackie virus type A.

Table 2 Sensitivities and specificities of antigen detection techniques for the diagnosis of adenovirus, HSV, and chlamydial keratoconjunctivitis in comparison with cell culture isolation

Causative agent	Test format	Commercial name	Sensitivity (%)	Specificity (%)	Reference	
Adenovirus	EIA	Cambridge Adenoclone	80.6	100	42	
			62.3	100	43	
		Cambridge Adenoclone	77	100	44	
		Cambridge Adenoclone	38	100	45	
		Cambridge Adenoclone	50.5	100	46*	
	IC	SAS Adenotest	54.7	97.1	46*	
	IF		79	100	42	
	IDB			85.3	92.2	47
				67	93	48
	HSV	IIF		97	73	49
DIF		Syva Microtrak	86	100	50†	
EIA		Kodak Surecell	37.5	81.3	51	
		Kodak Surecell	27	80	52	
		DuPont	65	100	53	
		Herpcheck				
			26	90	53	
<i>C trachomatis</i>	IF		59	100	53	
	DIF	Syva Microtrak	100	99	54	
			100	97.5	55	
		Syva Microtrak	57	81	56	
		Syva Microtrak	83.3	96.6	57	
			52	98	29	
			100	52	34	
	IPO		54	96	29	
	EIA	Abbott Chlamydiazyme	71	97	56	
		Abbott Chlamydiazyme	88	99	58	
Kodak Surecell		40	100	59		
		92.64	94.54	60‡		

DIF=direct immunofluorescence; IPO=immunoperoxidase; EIA=enzyme immunoassay; IIF=indirect immunofluorescence; IF=immunofiltration; IC=immunochromatography; IDB=immune dot blot.

\*Compared with PCR.

†Compared with clinical diagnosis.

‡Compared with DIF.

using various chemical or immunological staining techniques<sup>31-33</sup>; examination of blood and/or tears for various classes of antibodies<sup>34-41</sup>; and detection of viral or chlamydial antigens in conjunctival and corneal specimens<sup>29 34 42-60</sup> (Table 2). However, the pitfalls of these conventional techniques are numerous. Traditional cytological investigation is insensitive and subjective.<sup>28 29</sup> Cell culture isolation requires viable organisms necessitating special transport media and prompt transport of specimens 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 characterisation.

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 requirement 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 gold standard test used (culture 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 enzyme 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, Q $\beta$  replicase amplification, cycling probe reaction, strand displacement amplification, and branched DNA signal amplification.<sup>61</sup> 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<sup>62 63</sup> and since its first use in eye disease in 1990,<sup>64</sup> the test is now being widely used in clinical ophthalmology.<sup>65 66</sup> 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<sup>21 57 67-97</sup> (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 sensitive, accurate, and rapid than culture for detecting and typing adenovirus in cases of conjunctivitis.<sup>67 68</sup> In addition, PCR appears to be more sensitive than immunoassay in detection of ocular adenovirus infections. For example, Kington *et al*<sup>69</sup> evaluated the application of PCR in 107 ocular 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 immune dot-blot test.<sup>70 71</sup> 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.<sup>72</sup>

#### 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 culture 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.<sup>21 73-75</sup> Yamamoto *et al*<sup>73</sup> 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*<sup>21</sup> utilising cell culture as a gold standard test, PCR was shown to be

Table 3 Applications of PCR in ophthalmic infectious diseases

Causative agent	Ocular site	Clinical diagnosis	Reference
<b>Viruses:</b>			
Adenovirus	Conjunctiva	KC	67–72
HSV	Tear film	keratitis	73, 74
	Cornea or conjunctiva	keratitis or KC	21, 75, 76
EV70	Aqueous	uveitis	77, 78
	Conjunctiva	AHC	79
VZV	Aqueous and vitreous	retinal necrosis	78
	Vitreous	ophthalmic zoster	80
EBV	Aqueous and vitreous	retinal necrosis	81
	Vitreous	Vogt–Koyanagi–Harada syndrome	82
CMV	Aqueous, subretinal, and vitreous	chorioretinal inflammatory disease or retinitis	80, 83, 84
HPV	Conjunctiva	conjunctival carcinoma	85
<b>Bacteria:</b>			
<i>C trachomatis</i>	Conjunctiva	chlamydial conjunctivitis	56, 86–95
Mycobacteria	Vitreous and uveal tissue	endophthalmitis	96
<b>Parasites:</b>			
<i>Toxoplasma gondii</i>	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.

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.<sup>57–86–95</sup> Bobo *et al*<sup>84</sup> 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*<sup>90</sup> 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 test 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*<sup>93</sup> 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. Haller *et al*<sup>87</sup> evaluated the use of PCR, direct immunofluorescence, and serology for the detection of chlamydial IgA and IgG in 93 patients with follicular conjunctivitis. In comparison with cell culture, the sensitivities of PCR, direct immunofluorescence, and the three assays for IgA or IgG were 100%, 83.3%, and 62.5%–75% or 37.5%–100%, respectively, with specificities of 100%, 96.6%, and 70.6%–81% or 47.3%–64.8%, respectively. In a study of 75 cases of neonatal conjunctivitis, a commercially available PCR assay, Roche Amplicor, was found to be equivalent to culture for conjunctival specimens with sensitivity, specificity, and positive and negative predictive values of 92.3, 100, 100, and 98.4%, respectively.<sup>95</sup> PCR is a valuable epidemiological tool for detecting and genotyping ocular strains of *C trachomatis* from cases of inclusion conjunctivitis and trachoma.<sup>87–89</sup>

#### RELIABILITY

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.<sup>98</sup> 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.<sup>99</sup> 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 molecules<sup>100</sup> or by amplification of human DNA in the sample (for example,  $\beta$  globin gene).<sup>76</sup>

#### 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 system<sup>101</sup> and the ligase chain reaction test kit (Abbott Diagnostics Ltd).<sup>102</sup> While commercial test procedures and automation are, in relation to ocular disease, currently only available for *C trachomatis*,<sup>62–95</sup> 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.<sup>103</sup> 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 keratoconjunctivitis<sup>27</sup> 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<sup>46</sup> 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 (immunoassay). The current sophistication of PCR means that provided due attention is given to intralaboratory quality control including rigorous application of anti-cross contamination procedures,<sup>98</sup> 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 lead to the wider availability of quantitative procedures which will refine our ability to monitor the efficiency and progress of therapeutic regimens.

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.<sup>104-107</sup> In our own laboratory, for example, a multiplex PCR has been developed for the simultaneous investigation of adenovirus, HSV, and  $\beta$  globin in ocular swabs<sup>76</sup> 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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