PROSPECTIVE STUDY OF ADENOVIRUS ANTIGEN DETECTION IN EYE SWABS BY RADIOIMMUNE DOT-BLOT

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SUMMARY

Rapid laboratory diagnosis of ocular adenovirus infection is crucial in the containment of nosocomial transmission of the virus. In a large prospective study of adenovirus assay in eye swabs, antigen detection by radioimmune dot-blot (turnaround time 72 hours) achieved a sensitivity of 67% (239/355) and a specificity of 93% (3065/3285) in comparison with virus culture (median turnaround time 14 days). When specimens weakly reactive for adenovirus antigen, or equally reactive for both adenovirus antigen and *Chlamydia trachomatis* antigen, were considered falsely reactive in the adenovirus test, the sensitivity of the latter was reduced and false positive reactions were only marginally less frequent. The radioimmune dot-blot provides a more rapid diagnosis of ocular adenovirus infection than virus culture, but the high risk of false negative and in particular false positive results limits its clinical utility.

Adenovirus eye disease manifests as epidemic or sporadic keratoconjunctivitis, follicular conjunctivitis or pharyngoconjunctival fever.[1] Epidemic disease, associated with serotypes 8, 19 and 37 of subgenus D, can occur in large outbreaks in eye hospitals.[1,2] Sporadic cases are caused by a variety of adenovirus serotypes, including 3, 7, 14 (subgenus B), 1, 2, 5, 6 (subgenus C), 9, 10 (subgenus D), or 4 (subgenus E).[1]

Laboratory diagnosis of ocular adenovirus infection relies on antibody assay in serum, virus isolation in cell culture from eye swabs, or viral antigen detection in similar specimens by enzyme-linked immunosorbent assay.[1,3,4] The first two methods are retrospective, virus isolation taking a mean of 14 days. Moreover, given the large numbers of adenovirus serotypes, neutralising or haemagglutinating antibody tests can only be used if the likely infecting serotype is known.[1] We recently described an adenovirus antigen detection assay which provided a rapid result (within 72 hours), and achieved a sensitivity of 85% in a preliminary study.[5] We now report a large prospective evaluation of this radioimmune dot-blot test (IDBT) on 3760 conjunctival swabs.

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MATERIALS AND METHODS

All conjunctival swabs received between January 1990 and September 1993 were included if they had been tested for adenovirus by both IDBT and virus isolation. Cotton-tipped swabs were placed in 3 ml transport medium (Hanks' balanced salt solution with 10% [v/v] fetal calf serum, 2.5% [w/v] sodium bicarbonate, 200 international units/ml penicillin G, 200 mg/ml streptomycin and 5 mg/ml amphotericin B), and transported to the laboratory at ambient temperature.

Virus isolation on monolayer cultures of Hep-2, Vero and human embryo lung fibroblasts, and adenovirus typing by neutralisation, were done as previously described.[5]

The adenovirus IDBT was performed approximately twice weekly on residual samples stored at 4 °C.[5] Aliquots (0.2 ml) were heat-inactivated (56 °C for 30 minutes) and then dot-blotted in duplicate onto a pre-moistened nitrocellulose membrane. After blocking with 5% skimmed milk in phosphate-buffered saline (PBSM), the membrane was probed with PBSM containing 50–70 $\times 10^3$ counts/min/ml ^{125}I-labelled mouse monoclonal antibody specific for a genus reactive epitope on the adenovirus hexon protein.[6] Binding of the monoclonal antibody was revealed following autoradiography (48 hours at −70 °C). To check the specificity

Table I. Adenovirus positivity rates in cell culture for eye swabs showing various combinations of reactivity in the adenovirus and chlamydial IDBT

Adenovirus IDBT[a]	Chlamydial IDBT[a]	Proportion of adenovirus positive in virus isolation
+	−	90/135 (67%)
+	±	38/62 (61%)
++	+	39/50 (78%)
±	−	42/94 (45%)
±	±	19/81 (23%)
+	+	10/24 (42%)
±	+	1/13 (8%)

[a]Results on autoradiograph were scored as: −, negative; ±, weak positive (incomplete circle or circular darkening less intense than control for the same antigen); +, positive (dark circle at least as intense as the control for the same antigen); ++, strong positive (dark circle more intense than the control for the same antigen and more intense than the circle for the other antigen).

of any reaction in the adenovirus IDBT, the conjunctival swab transport medium was also tested for *Chlamydia trachomatis* lipopolysaccharide antigen using an IDBT identical in all respects to the adenovirus assay except for reliance on a *Chlamydia*-specific mouse monoclonal antibody.[7] Complete darkened circles on the autoradiograph were considered to indicate reactivity with the relevant antibody. In each assay doubling dilutions of a positive control were included: either adenovirus type 2 infected Hep-2 cells or *Chlamydia trachomatis* infected McCoy cells. A sample was considered reactive if the circle it produced was greater in intensity than the circle which corresponded to 25 tissue culture infectious dose – 50 of adenovirus type 2[5] or 100 inclusion-forming units of *Chlamydia trachomatis*.[8] Incomplete circles or circles of lower intensity than these controls were interpreted as weakly positive reactions. If both reactions were positive, the more intense was classified as strongly positive.

Statistical analysis relied on chi-squared or Fisher's exact tests, as appropriate, p values being two-tailed.

RESULTS

Eye swabs yielding herpes simplex virus (n=119) or varicella zoster virus (n=1) in cell culture were excluded from further analyses. Of the 239 adenovirus IDBT and culture positive specimens, 62 (26%) reacted only weakly for adenovirus antigen, and 107 (45%) reacted simultaneously for adenovirus and *Chlamydia trachomatis*. Compared with virus isolation, the adenovirus IDBT achieved a sensitivity of 67% (239/355), a specificity of 93% (3065/3285), a positive predictive value of 52% (239/459) and a negative predictive value of 96% (3065/3181) if all reactivity for adenovirus antigen of this IDBT was considered to reflect the presence of that antigen. If the specimens producing weakly reactive results in the adenovirus IDBT were considered negative for adenovirus antigen the sensitivity of the rapid assay was lower than before (177/355, 50%; p<0.001), the positive predictive value (177/271, 65%) increased (p<0.001), but the specificity (3127/3285. 95%) and the negative predictive value (3127/3305, 95%) were virtually unchanged. If a specimen was considered positive for adenovirus antigen only when it reacted more intensely with anti-adenovirus antibody than with the chlamydial antibody, the sensitivity was again reduced (209/355, 59%; p = 0.008), the positive predictive value (209/341, 61%) increased (p<0.01), and there was little effect on the specificity (3095/3285, 94%) and the negative predictive value (3095/3241, 95%).

Specimens more strongly reactive in the adenovirus than the chlamydial antigen test were more likely to be positive for adenovirus in cell culture than were specimens equally reactive in the two tests or more strongly reactive for chlamydial antigen (209/341 [62%] versus 30/118 [25%], p<0.001) (Table I). When all reactivity with anti-adenovirus antibody was considered positive and the *Chlamydia* result was not taken into account, the sensitivity of the adenovirus IDBT was lower with subgenus C adenovirus strains than with other typed adenoviruses (Table II) (p<0.001), with specimens positive for serotype 7 (p=0.03) or 8 by virus isolation (p=0.009), and possibly with specimens similarly positive for adenoviruses of subgenera B (p=0.06) or E (p=0.07). Low sensitivity was seen with all four adenovirus subgenus C serotypes (Table II).

DISCUSSION

Overall the performance of the adenovirus IDBT was disappointing, with a sensitivity of only 67% in comparison with the 85% reported previously from our laboratory.[5] Other workers reported a sensitivity for antigen detection by enzyme-linked immunosorbent assay of only 75% in comparison with virus isolation.[3,4] Differences between our initial and present studies included a change in the temperature at which specimens were stored prior to antigen testing (−40 °C versus 4 °C), and doubling of the

Table II. Adenovirus subgenera and serotypes and sensitivity of adenovirus IDBT in comparison with virus isolation

Subgenus	Serotype	Sensitivity
B	3, 7, 14	92/134 (69%)
	3	62/95 (65%)
	7	28/36 (78%)
	14	2/3 (67%)
C	1, 2, 5, 6	13/26 (50%)
	1	5/11 (45%)
	2	5/9 (56%)
	5	1/2 (50%)
	6	2/4 (50%)
D	8, 9, 10	62/95 (65%)
	8	24/28 (86%)
	9	0/1 (0%)
	10	38/66 (58%)
E	4	47/66 (71%)
Untyped		25/34 (74%)

Chlamydia IDBT results were disregarded in this analysis.

transport medium volume from 1.5 to 3.0 ml to guarantee an adequate volume for all tests. We did not determine whether either of these alterations reduced the assay sensitivity. The sensitivity in the current study was probably a more accurate reflection of the test performance than the earlier results, given the difference in specimen numbers (3760 versus 754). Only in the larger study was a subgenus-specific difference in IDBT performance identified, with low sensitivity for subgenus C strains but excellent sensitivity for type 8 strains. This difference could have reflected the presence of differing amounts of viral antigen in the conjunctival secretions of patients infected with adenoviruses of different subgenera. The relatively high specificity reported earlier (92%)[5] was maintained (93%), but the low positive predictive value compromised the overall clinical usefulness of the test even when weak reactivity for adenovirus antigen was considered false. Nevertheless, the adenovirus IDBT proved valuable in containing a recent outbreak of adenovirus type 8 keratoconjunctivitis.[9]

Immune dot-blots where the total protein in a sample is immobilised on a nitrocellulose membrane may give false positive results when specimens contain staphylococcal protein A.[7] Specimen pretreatment with proteinase K circumvented this problem in an IDBT for *Chlamydia trachomatis* lipopolysaccharide antigen,[8] but could not be applied in the adenovirus assay since a protein antigen was assayed. In a previous report we recommended simultaneous testing of eye swabs for adenovirus and either chlamydial or herpes simplex virus antigens to differentiate true positive reactivity (positive result only for adenovirus) from false reactivity (positive results for both antigens).[5] In the present study such an approach led to a reduction in the sensitivity of the adenovirus IDBT, and increased its positive predictive value to only 61%. Specimens selectively reactive for adenovirus antigen were more likely to yield adenovirus in cell culture than those equally reactive for both adenovirus and chlamydial antigens, but 8–42% of specimens in the latter category (depending on the individual antigen results; Table I) yielded adenovirus in virus isolation.

To determine the significance of discrepant results in the adenovirus and chlamydial antigen detection and adenovirus culture tests, adenovirus DNA was sought in a small number of specimens using a genus-reactive polymerase chain reaction (PCR).[10] Adenovirus DNA was detected by PCR in 0 of 6 specimens reactive in both IDBTs, and in 3 of 7 specimens reactive only in the adenovirus antigen assay when virus isolation gave a negative result (A. S. Bailey, unpublished observations). The PCR result therefore suggested that even a positive reaction in the adenovirus IDBT and a negative result in the *Chlamydia* assay did not always indicate the presence of adenovirus in the clinical specimen. However, the specimens equally reactive in both IDBTs or more strongly reactive in the chlamydial test were not all falsely positive in the adenovirus antigen assay, as adenoviruses were detected in cell culture from 30 of 118 such specimens.

In the late 1980s we developed two assays intended *inter alia* to improve the diagnosis of ocular infection. Assay of *Chlamydia trachomatis* lipopolysaccharide antigen proved more sensitive than chlamydial culture, and antigen-positive culture-negative specimens mostly contained chlamydial DNA detectable using the PCR.[11] In contrast the adenovirus antigen detection assay was less sensitive than cell culture and generated both uninterpretable low positive and apparently false positive results. This difference could in part have reflected differences in the amount of antigen in eye swabs during the two infections. More importantly, however, the restriction of proteinase K specimen pretreatment to the chlamydial assay was crucial because this increased both sensitivity and specificity.[8] Use of Fab_2 fragments of the adenovirus monoclonal antibody for radiolabelling might have increased the specificity of adenovirus assay because protein A binds to the Fc portion of immunoglobulin.[12] However, our unpublished observations indicated that digestion of this antibody with pepsin resulted in poor yield of antibody, and that the resultant Fab_2 fragment was inactivated on radioiodination. Presumably the tyrosine residue enabling radioiodination of the adenovirus IgG monoclonal antibody with retention of biological activity resides on the Fc portion of antibody molecule. Attempted circumvention of this problem through the use of non-isotopic labelling (biotin-avidin labelling with dye precipitation: 5-bromo-4-chloro-2-indolyl phosphate dye) did not produce an assay of adequate sensitivity and specificity.

Chlamydia antigen detection using the IDBT has an established place in the diagnosis of ocular infection,[11] but the role of adenovirus antigen detection by IDBT is less certain. We and others have recently reported that DNA amplification using the PCR is more sensitive than antigen assays for the detection of adenoviruses in eye swabs.[13,14] The former technique may generate false positive results due to specimen contamination with exogenous adenovirus DNA or reaction product.[13] The present study nonetheless indicates that the IDBT has such a low sensitivity and such a high false positivity rate that its replacement by the PCR for the rapid diagnosis of ocular adenovirus infection is inevitable.

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Key words: Adenovirus, Antigen detection, Conjunctivitis, Dot-blot test, Rapid diagnosis.

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