Comparison of methods for the laboratory diagnosis of ocular adenovirus type 3 infection

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In this volunteer study of an induced adenovirus type 3 infection, fluorescent antibody staining, and the examination of conjunctival cytology by Giemsa and Papanicolaou stains were not diagnostically useful. Virus isolations and antibody rises were the only suitable laboratory indications of infection.

Key words: adenovirus infections, adenovirus group, fluorescent antibody technique, conjunctiva, virus isolation, antibody, negative study.

At present, the laboratory confirmation of clinical adenovirus infection is generally made by either the isolation of virus in tissue culture or the finding of rising antibody titers. Since it may take as long as two months to isolate an adenovirus and type it, and since the determination of rising titers is also laborious, it was hoped that some other method might be found for the rapid and reliable identification of adenovirus infection.

The assessment of diagnostic techniques for any disease is often difficult because evaluation of sensitivity requires the investigator to know the number of people actually infected. In this study, a group of volunteers were infected with a known type of adenovirus, adenovirus type 3, and several diagnostic techniques were compared. The first involved the use of fluorescent antibody staining of conjunctival scrapings. The use of such staining has previously been reported, but it has never been evaluated in an acceptable way to determine its diagnostic sensitivity. This study seemed ideal since the type of adenovirus was certain and specific antibody was available in high concentrations. Standard Giemsa and Papanicolaou stains of conjunctival scrapings were similarly evaluated to determine whether the typical intranuclear inclusion seen in adenovirus-infected cells in tissue culture might be visible in clinical studies in a significant proportion of those infected. These techniques were then compared with virus isolations.

Methods and materials

A total of 160 male subjects at the Florida Division of Correction, Raiford, Fla., were tested for....

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specific neutralizing antibodies to adenovirus type 3. Of these, only 28 were seronegative; these seronegative subjects, as well as two who had very low antibody levels (1:10), were included in the study. The 30 subjects were infected bilaterally with adenovirus type 3 by instilling two drops of the virus suspension into the lower conjunctival sac. The virus used was isolated at the University of Florida on primary human amnion tissue cultures from a young male patient with pharyngoconjunctival fever. It was passed once in this tissue and stored at -70° C. prior to use.

Each subject was examined daily for two weeks and twice a week for two more weeks. Temperature, pharyngitis, and cervical and preauricular adenopathy were recorded and lid reaction, conjunctival follicular hypertrophy, hyperemia, and corneal changes were studied by slit lamp biomicroscopy and were photographed. Subjects were questioned daily for general symptoms of malaise, nausea, vomiting, abdominal pain, diarrhea, sore throat, cough, sputum, muscle ache, skin rash, and also for ocular symptoms of pain, foreign body sensation, discharge and lid stickiness, photophobia, tearing, and blurred vision. Investigators did not have access to the previous

![Graph 1](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933002/)

**Fig. 1.** The severity of disease for several of the volunteers was averaged to give a general idea of the course of infection. The virus isolations were most frequent when the disease was most severe and were less frequent as the disease waned. Fluorescent antibody staining was positive primarily before disease was clinically apparent.

![Graph 2](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933002/)

**Fig. 2.** Although there is not a good correlation, in general, virus was cultured more frequently from the volunteers who had more severe infections.
day’s findings during their observations and questioning of subjects.

Conjunctival reaction, follicular hypertrophy, adenopathy, and pharyngitis were graded on the basis of severity so that 4 was the most severe reaction possible and 0 was no reaction at all.

Although the subjects were divided into three groups for treatment purposes, no treatment was effective, there was no difference between groups, and all results will be considered together.3

Virus cultures were made by swabbing the lower conjunctival cul-de-sac and the pharynx with dry cotton swabs. The swabs were placed in tissue culture medium and frozen at -70° C. until later in the day when the medium was placed on primary human amnion tissue cultures. The cultures were observed for CPE for three weeks, then discarded.

Scrapings of the conjunctival epithelium were made from alternate eyes every day for fluorescent antibody and staining with Ciemsa and Papanicolaou’s stains. The smear for fluorescence testing was fixed immediately in cold acetone for ten minutes, air dried, and then frozen at -70° C. until stained. The majority of smears were stained the day after collection; the rest within five days. After thawing, the slides were incubated with specific rabbit antiadenovirus type 3 serum for 30 minutes at 37° C, rinsed in phosphate-buffered saline, and reincubated with fluorescein-conjugated goat antirabbit serum.

Results

All subjects became clinically diseased three days after infection; after 10 to 14 days, signs and symptoms had disappeared. In the majority of cases, disease consisted of mild conjunctival hyperemia, pharyngitis, moderate conjunctival hypertrophy, and preauricular adenopathy. Only one subject had a transient fever. There were usually mild photophobia, tearing, and foreign body sensation.

No positive cultures were obtained until 48 hours after infection, or the day before the subjects became clinically diseased; none were obtained after the twelfth day. Positive cultures were obtained sporadically during this time. The number of positive isolations per individual did not correlate with the severity of disease; however, the majority of isolations were made at the time disease was at its peak and became less as disease regressed (Figs. 1 and 2). More positive cultures were obtained from the eye than from the pharynx (Table I). Five individuals never had any positive cultures, even though they had mild disease and developed antibodies to adenovirus type 3, but 83 per cent of the subjects had a positive culture at some time.

Positive fluorescence was seen in 11 of the 30 scrapings of conjunctival epithelium taken on the day after infection. Of the remaining 690 scrapings, only three others were positive after that, one on the next day and two on the eighth day after infection.

Although 840 smears were stained by the Giemsa stain and 840 by Papanicolaou’s stain, not one easily diagnosable adenovirus inclusion was seen.

Discussion

It was clear from the study that the most reliable way to diagnose adenovirus infections is by virus culture. Attempts to provide a more rapid diagnosis by using fluorescent antibody techniques and conjunctival cytology were not successful, even in this situation in which the precise type of adenovirus infection was known, and the exact fluorescent antibody serum could be used.

It was somewhat surprising that the detection of adenovirus infection by fluorescent antibody staining was possible only immediately after infection. By the time symptoms had developed, there were virtually no positives by fluorescent antibody

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Table I. Viral isolations and conjunctival epithelium staining for first 12 days after infection

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<tr>
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<th>Number positive total taken</th>
<th>Per cent positive</th>
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<tbody>
<tr>
<td></td>
<td>Eye</td>
<td>Pharynx</td>
</tr>
<tr>
<td>Positive virus isolations</td>
<td>120/720</td>
<td>18/360</td>
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<tr>
<td>Positive fluorescent antibody staining</td>
<td>14/720</td>
<td>—</td>
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<tr>
<td>Giemsa and Papanicolaou’s stains</td>
<td>0/1,680</td>
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stainings. On the contrary, virus isolation was most likely to be positive at the time when the patient was symptomatic, and tended to be negative during the early phases of fluorescent antibody staining. Although the interpretation of these results is not completely clear, they suggest that the initial infection of the epithelial cell in the presymptomatic phase of disease permitted positive fluorescent antibody stains, even before virus multiplication had become a prominent part of the disease. The finding of virus at a later date, however, when epithelial cells were negative by fluorescent antibody staining, suggests that the primary site of virus multiplication during the symptomatic phase of the disease may not be the conjunctival epithelium, but may be tissue beneath the epithelium—either lymphocytes or the substantia propria of the conjunctiva. These relatively poor results differ from those of Uchida and colleagues who, using a direct fluorescent antibody stain in patients with clinical EKC, found "about 50 per cent" positive during the first two weeks of disease. The direct-staining technique and the frequency of type 8 adenovirus might contribute to a greater diagnostic efficiency, but the tendency toward false positives with this technique which is easily "over-read" cannot be evaluated. No detailed data are presented in Uchida's EKC study which would permit a comparison of clinical diagnoses with virus isolation and positive fluorescence in individual cases, but in the present study, virus isolation was positive in 83 per cent of the patients. The difficulty in finding typical intranuclear inclusions by standard cytological techniques in conjunctival scrapings further suggests that the method may be adequate but that, in this case, the virus may be multiplying primarily in other cells.

REFERENCES