

NOTES

Immunochromatography Test for Rapid Diagnosis of Adenovirus Respiratory Tract Infections: Comparison with Virus Isolation in Tissue Culture

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The sensitivity and the specificity of a new commercial rapid 10-min adenovirus antigen immunochromatography (IC) test were determined by comparison with the sensitivity and specificity of virus isolation. Of 169 pharyngeal swabs from children with suspected adenovirus respiratory tract infections, 95 (56%) were culture positive for adenovirus. The IC test was sensitive (detecting 69 of these 95 infections [72.6%]) and completely specific (identifying 74 of 74 specimens [100%]) when it was compared with cell culture. The test detected adenovirus serotypes 1, 2, 3, 5, and 7 with almost equal sensitivities. This test is not only rapid and easy to perform but also sensitive and specific for adenovirus respiratory tract infections. The test is sufficiently rapid to be used at the bedside or in an outpatient clinic, with the result being available during a patient's first examination.

Adenovirus is a leading cause of viral infection in children and has been implicated in a wide range of clinical diseases affecting mainly the respiratory, ocular, and gastrointestinal systems (3). Respiratory tract adenovirus infection manifests itself in various clinical forms, including pharyngitis, exudative tonsillitis, pharyngoconjunctival fever, and pneumonia (3). Many of these infections are difficult to distinguish clinically from other respiratory virus infections and some bacterial infections. Laboratory diagnosis such as by cell culture and viral serology is usually necessary to identify the etiologic agent (5, 7).

Final results of adenovirus isolation by tissue culture usually require several days. A more rapid diagnosis can be made by direct detection methods, such as enzyme immunoassay, radioimmunoassay, and direct immunofluorescent antibody techniques, with pharyngeal or conjunctival epithelial cells (1, 4, 7–9). Although these tests are moderately sensitive in detecting adenovirus in respiratory tract specimens, they require special equipment, take at least 1 h to complete, and, consequently, are not ideal for wider clinical application at the bedside or in an outpatient clinic.

We evaluated a new rapid diagnostic test, the adenovirus antigen immunochromatography (IC) test (SA Scientific Inc., San Antonio, Tex.), for its clinical usefulness in detecting adenovirus antigen in pharyngeal specimens from subjects with respiratory tract infections. This test takes 10 to 15 min to

perform and relies on a monoclonal antibody that binds to a group-reactive hexon antigen common to 49 known human adenovirus serotypes. The sensitivity, specificity, and convenience of the test were assessed.

One hundred sixty-nine patients less than 15 years old were included; 45, 87, and 36 subjects were diagnosed as having pharyngitis, exudative tonsillitis, and pharyngoconjunctival fever, respectively. The patients were seen during 1997 to 1998 at six institutions. Pharyngeal swabs were obtained from each subject with two cotton tips. One swab was inoculated directly into a human foreskin cell culture bottle for virus isolation, and the other was placed in 500 μ l of 10 mM Tris-HCl (pH 8.0)–1 mM EDTA for the IC test.

For virus isolation, the specimens were monitored daily for 4 weeks for the appearance of a cytopathic effect. Isolates were identified as adenovirus by an immunofluorescence test with a polyclonal antibody which reacts to all known human adenovirus serotypes. The serotype of each isolate was determined by serum neutralization tests.

The adenovirus antigen IC test was performed according to the manufacturer's directions. The test is a sandwich immunoassay that uses a paper membrane with a monoclonal antibody in the liquid phase and two polyclonal antibodies in the solid phase. The liquid-phase antibody is a gold colloid-conjugated mouse monoclonal antibody to adenovirus capsid hexon (signal antibody), while the two solid-phase antibodies are a polyclonal antibody to adenovirus and a polyclonal antibody to mouse immunoglobulin. The signal-antibody segment is adjacent to the round well of the sample aliquot. Briefly, the 10-min, one-step procedure is as follows: 200 μ l of a pharyngeal swab specimen is transferred to the round well of the testing device. The specimen migrates via capillary action along the

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TABLE 1. Comparison of IC test with cell culture in adenovirus detection

Adenovirus culture result	No. of specimens with IC test result		Total no. of specimens
	Positive	Negative	
Positive	69	26	95
Negative	0	74	74
Total	69	100	169

membrane, and adenovirus reacts with the signal antibody. Adenovirus-signal antibody complex also reacts with the polyclonal antibody to adenovirus and forms a colored line that develops within 10 min. The excess signal antibody which does not bind to adenovirus migrates further until it reacts with the polyclonal antibody to mouse immunoglobulin, producing a separate, second colored line. Thus, two colored lines on the test stick indicate the presence of adenovirus hexon antigen. In the absence of adenovirus, only one colored line develops, as a result of the reaction between the signal antibody and the antibody to mouse immunoglobulin.

The significance of the differences in sensitivity of the IC test between samples obtained early and later in the disease was determined by the χ^2 test.

Of 169 samples, 95 (56%) were positive for adenovirus by tissue culture; 6 (6.3%), 13 (13.7%), 70 (73.7%), 3 (3.2%), and 3 (3.2%) of the isolates were identified as adenovirus types 1, 2, 3, 5, and 7, respectively. Of the 95 adenovirus strains, 12, 57, and 26 were isolated from patients with pharyngitis, exudative tonsillitis, and pharyngoconjunctival fever, respectively. The cell culture and IC test results are summarized in Table 1. The IC test was highly specific (identifying 74 of 74 specimens [100%]) and also sensitive (detecting 65 of the 95 culture-positive specimens [72.6%]) compared with the results of cell culture.

The test detected adenovirus serotypes 1, 2, 3, 5, and 7 with almost equal sensitivities, that is, at around 70% (Table 2).

The earlier specimens were tested, the higher the sensitivity obtained. The positive rate of the IC test for specimens obtained within 4 days of the onset of illness (45 of 56 specimens [80.4%]) was significantly higher than that for specimens obtained 5 to 11 days after the onset of the illness (24 of 39 specimens [61.5%]) (χ^2 test, $P < 0.05$) (Table 3).

Differentiation of bacterial from viral infection is a common clinical problem. With adenovirus, respiratory tract infections in children are often characterized by high-grade, prolonged fever and by abnormal laboratory findings such as neutrophilia and elevated levels of acute-phase reactants. These findings are also consistent with those for bacterial infections (6). Con-

TABLE 2. Sensitivity of IC test by adenovirus serotype

Adenovirus serotype	No. of specimens positive by:		% Sensitivity of IC test
	Culture	IC test	
1	6	4	66.7
2	13	9	69.2
3	70	52	74.3
5	3	2	66.7
7	3	2	66.7
Total	95	69	72.6

TABLE 3. Effect of the amount of time after the onset of adenovirus diseases upon sensitivity of the IC test

No. of days specimen taken after onset	No. of positive specimens by:		% Sensitivity of IC test
	Culture	IC test	
1–4	56	45	80.4 ^a
5–11	39	24	61.5 ^a
Total	95	69	72.6

^a $P < 0.05$ (χ^2 test).

sequently, a simple, sensitive, and rapid diagnostic test for adenovirus infections would be invaluable to those caring for children. Rapid confirmation of adenovirus would allow a pediatrician to counsel a child's parents about the prognosis and to give specific advice to restrict further transmission of the virus. Furthermore, it would also eliminate unnecessary antibiotic use for suspected streptococcal or other bacterial infections (2, 3).

In this study, we demonstrate the clinical usefulness of a rapid, one-step IC test for the diagnosis of adenovirus respiratory tract diseases. When compared to the sensitivity of adenovirus isolation in cell culture, the sensitivity of this test exceeded 70% while the specificity was absolute (100%). When specimens were obtained within 4 days of the onset of illness, the sensitivity exceeded 80%. In a study using conjunctival specimens from subjects with pharyngoconjunctival fever, this IC test had a sensitivity of approximately 50% when it was compared to an adenovirus PCR method (10). The lower sensitivity in that report might be partially due to the comparison with PCR, which is generally more sensitive than culture in a clinical setting.

Immunofluorescent staining of exfoliated pharyngeal epithelial cells has also been employed for the rapid diagnosis of adenovirus infections (4). This technique is reported to have a sensitivity similar to that of the IC test but depends on the skill of the microscopist and the availability of a fluorescence microscope (8, 9). A recently introduced enzyme-linked immunosorbent assay kit for the detection of adenovirus in pharyngeal or conjunctival swabs (Adenoclone, Cambridge, Mass.) may be superior to immunofluorescent staining, because no specific skill or special instruments are needed. The Adenoclone test detects adenovirus antigen with sensitivities of 38 and 73% compared to the sensitivities of tissue culture with conjunctival and pharyngeal specimens, respectively (2, 11). Its results with pharyngeal samples almost equals those by the IC test, but the Adenoclone test requires at least 90 min to complete, which creates a difficulty in adoption of this kit for the diagnosis of adenovirus respiratory infection as part of an initial examination.

In contrast, the IC test can be completed within 15 min with a high sensitivity and specificity and without special instruments. The IC test provides rapid helpful information for diagnosis and for developing a treatment plan for patients with suspected adenovirus respiratory diseases. These results can be available during the patient's first examination, at the bedside, or in an outpatient clinic.

REFERENCES

- August, M. J., and A. L. Warford. 1987. Evaluation of a commercial monoclonal antibody for detection of adenovirus antigen. *J. Clin. Microbiol.* **25**: 2233–2235.
- Hara, M., A. Tanabe, T. Saeki, H. Shimizu, R. Matsuura, A. Sakamoto, and E. Arihiro. 1996. Rapid diagnosis of adenoviral upper respiratory tract in-

- fections using enzyme immunoassay. *J. Jpn. Pediatr. Soc.* **100**:1181–1188. (In Japanese.)
3. **Horwitz, M. S.** 1996. Adenoviruses, p. 2149–2171. In B. N. Fields and D. M. Knipe (ed.), *Fields virology*, 3rd ed. Raven Press, New York, N.Y.
 4. **McCormick, D. P., Q. Galapon, and C. Berling.** 1972. Pathology of exfoliated oropharyngeal epithelial cells infected with wild-type adenovirus. *Appl. Microbiol.* **24**:389–397.
 5. **Putto, A.** 1987. Febrile exudative tonsillitis; viral or streptococcal? *Pediatrics* **80**:6–12.
 6. **Putto, A., O. Meurman, and O. Ruuskanen.** 1986. C-reactive protein in the differentiation of adenoviral, Epstein-Barr viral and streptococcal tonsillitis in children. *Eur. J. Pediatr.* **145**:204–206.
 7. **Ruuskanen, O., H. Sarkkinen, O. Meurman, P. Hurme, T. Rossi, P. Halonen, and P. Hanninen.** 1984. Rapid diagnosis of adenoviral tonsillitis: a prospective clinical study. *J. Pediatr.* **104**:725–728.
 8. **Takimoto, S., M. Grandien, M. A. Ishida, M. S. Pereira, T. M. Paiva, T. Ishimura, E. M. Makita, and C. H. Martinez.** 1991. Comparison of enzyme-linked immunosorbent assay, indirect immunofluorescence assay and virus isolation for detection of respiratory viruses in nasopharyngeal secretions. *J. Clin. Microbiol.* **29**:470–474.
 9. **Trabelsi, A., B. Pozzetto, A. D. Mbida, F. Grattard, A. Ros, and O. G. Gaudin.** 1992. Evaluation of four methods for rapid detection of adenovirus. *Eur. J. Microbiol. Infect. Dis.* **11**:535–539.
 10. **Uchio, E., K. Aoki, W. Saitoh, N. Itoh, and S. Ohno.** 1997. Rapid diagnosis of adenoviral conjunctivitis of conjunctival swabs by 10-min immunochromatography. *Ophthalmology* **104**:1294–1299.
 11. **Wiley, L. A., L. A. Roba, R. P. Kowalski, E. G. Romanowski, and Y. J. Gordon.** 1996. A 5-year evaluation of the Adenoclone test for the rapid diagnosis of adenovirus from conjunctival swabs. *Cornea* **15**:363–367.