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Evaluation of a Commercial Monoclonal Antibody for Detection of Adenovirus Antigen

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The Adenoclone monoclonal antibody for detection of adenovirus antigen was evaluated by enzyme immunoassay and a 72-h indirect fluorescent-antibody test in shell vials and compared with standard culture. The sensitivity and specificity of the Adenoclone test were both 100% with 114 cell culture isolates. With 310 direct clinical specimens, the Adenoclone enzyme immunoassay sensitivity varied from 14.3 to 66.6%, but the Adenoclone indirect fluorescent-antibody test sensitivity was 86.7%, with 100% specificity.

Techniques for adenovirus antigen detection, including radioimmune assay, DNA hybridization, enzyme immunoassay (EIA), and fluorescent-antibody stains, have been used successfully; however, only a polyclonal fluorescent-antibody reagent has been available for use in detection of adenovirus in the routine clinical laboratory (2, 5, 8, 9, 15). We evaluated a new commercial monoclonal antibody (Cambridge Bioscience, Hopkington, Mass.) in indirect fluorescent-antibody (IFA) tests and EIAs for detection of adenovirus antigen in direct specimens from patients and of cell culture isolates to improve the speed and accuracy of adenoviral diagnosis.

Specimens. The clinical specimens consisted primarily of conjunctival swabs from ophthalmology clinics and respiratory and fecal samples from pediatric patients or inpatients with acquired immunodeficiency syndrome. The conjunctival, rectal, and respiratory specimens were submitted in Transporter tubes (Bartels Immunodiagnostics, Bellevue, Wash.) of human fibroblasts in 2 ml of minimum essential medium as previously described (16). Stool samples were collected in sterile screw-cap containers. All specimens were transported in styrofoam containers on cold packs.

Standard culture method. A portion of fecal sample was diluted in 2 ml of phosphate-buffered saline (PBS) to approximate a 1:10 suspension. Both fecal suspensions and swabs in Transporter tubes were treated with 0.5 ml of antibiotic mixture as previously described (16). Treated specimens were inoculated into four cell culture tubes each (human foreskin, IMR-90, primary rhesus monkey kidney, and either Buffalo green monkey or rhabdomyosarcoma cells [Bartels Immunodiagnostics and Viromed Labs, Minneapolis, Minn.]) and two 1-dram (shell) vials with human foreskin fibroblast monolayers on cover slips. After inoculation, specimens were stored at either −70°C in a freezing medium consisting of minimum essential medium with 20% dimethyl sulfoxide and fetal bovine serum (Sterile Systems, Logan, Utah) or 1 to 4°C for 1 to 5 days for EIA testing. Culture tubes were refed 12 h after inoculation and weekly thereafter with minimum essential medium containing 2% fetal bovine serum, gentamicin sulfate (15 μg/ml), and amphotericin B (2.5 μg/ml). All inoculated cell culture tubes were examined microscopically on a daily basis for the first 7 days and three times per week from days 8 to 28 or until cytopathic effects (CPE) were detected. Cultures showing evidence of CPE were tested by scraping the cell monolayer and preparing slides stained with polyclonal antiadenovirus fluorescein isothiocyanate conjugate (PDFA; M.A. Bioproducts, Walkersville, Md.) according to manufacturer instructions after herpes simplex virus (HSV) was excluded with an enzyme-linked immunosorbent assay, the HSV Antigen ELISA (Ortho Diagnostics, Carpinteria, Calif.). For early (pre-CPE) detection of adenovirus in eye and stools specimens, human foreskin fibroblast-containing vials were acetone fixed and stained on days 5 to 7 with polyclonal antiadenovirus conjugate (PDFA) as described above.

EIA procedure. Treated specimens tested by EIA were stored after culture inoculation for up to 5 days at 1 to 4°C or frozen immediately in 1 ml of the freezing medium described above. Adenoclone microtiter wells (Cambridge Bioscience) coated with anti-adenovirus monoclonal antibody were inoculated in duplicate with 100 μl of refrigerated or freeze-thawed samples from patients and positive and negative controls. Negative controls consisted of PBS, transporter medium, dimethyl sulfoxide freezing medium, and antibiotic mixture. Cultures of adenovirus type 1 (American Type Culture Collection, Rockville, Md.) titrated to 100 50% tissue culture infective doses were used as positive controls. After specimen inoculation, the Adenoclone conjugate, consisting of horseradish peroxidase conjugated to antiadenovirus monoclonal antibody (100 μl), was added to all of the wells, and the plates were incubated for 60 min at room temperature. Microtiter wells were then aspirated and washed with deionized water by using a Behring ELISA Processor. Both hydrogen peroxide substrate and tetramethylbenzidine chromogen (100 μl) were added to each well, followed by 10 min of incubation at room temperature. To stop the reaction, sulfuric acid (1 N; 100 μl) was added to the wells, and the A_{450} was determined with the Behring ELISA Processor.

Specimens with A_{450}s of >0.150 were considered positive for adenovirus by EIA. All specimens with positive EIA results but negative for CPE or fluorescent-antibody testing were retested by all of the methods.

Monoclonal IFA procedure. Treated clinical samples or tissue culture isolates inoculated into shell vials were incubated at 36°C for 72 h. Monolayers were washed twice with 1-ml volumes of PBS, followed by acetone fixation for 10 min at room temperature. Acetone was aspirated, and 50 μl of antiadenovirus monoclonal antibody (Cambridge Bioscience) at a 1:1,000 dilution in PBS was delivered to each

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TABLE 1. Comparison of cell culture with the Adenoclone IFA test and EIA with clinical specimens

Culture result	No. of results by[a]:			
	72-h IFA test		Direct EIA	
	Positive	Negative	Positive	Negative
Positive	39	6[b]	25	19
Negative	2[c]	243[d]	2[c,e]	257

[a] The sensitivity and specificity of the IFA test were 86.7 and 100%, respectively, and those of the EIA were 56.8 and 100%, respectively.
[b] Two questionable results were excluded.
[c] Adenovirus types 40 and 41 were identified (pediatric stools).
[d] Fourteen vials were excluded for lack of cells.
[e] Three nonreproducible results were excluded.

cover slip, followed by 30 min of incubation at 36°C, a single PBS wash, and a further 30 min of incubation with 50 μl of conjugate (fluorescein-conjugated immunoglobulin G fraction of goat anti-mouse immunoglobulin G, heavy and light chain specific; Cooper Biomedical, Inc., West Chester, Pa.) diluted 1:100 in PBS. Optimum dilutions of antibody and conjugate were predetermined by box titration on adenovirus-infected control slides (Bion Enterprises, Park Ridge, Ill.). Unbound conjugate was removed with a final PBS wash, and cover slips were counterstained with Evans blue before mounting with buffered glycerol (Electro-Nucleonics, Inc., Columbia, Md.). Cover slips were examined for specific fluorescence by using a Zeiss fluorescence microscope with 10× and 25× objectives.

We tested 310 direct specimens from patients and 114 isolates from cell culture by EIA and the IFA test with the Adenoclone monoclonal antibody and by the standard method. No adenovirus was detected by any of the methods in 259 of the specimens from patients tested (Table 1).

The 45 specimens positive by all three methods consisted of 29 conjunctival swabs (predominantly adenovirus types 8 and 11), 1 nasopharyngeal swab, 1 rectal biopsy, and 14 stool samples. One conjunctival specimen was positive by PDFA but negative by culture, EIA, and the monoclonal IFA test. Two pediatric stool specimens were positive with the Adenoclone EIA and the monoclonal IFA test but did not develop CPE in 28 days. These specimens were positive with monoclonal antibody specific only for adenovirus types 40 and 41 by the IFA test and EIA (Cambridge Bioscience) (Table 1).

The average time to detection of CPE for the 45 culture-positive specimens was 9.5 days (range, 2 to 28 days). Of these adenovirus isolates, 35 (78%) were also detected in 5 to 7 days with the PDFA test. Of these 35, 16 were PDFA positive before detection of CPE, and 19 developed CPE before day 5. The PDFA test was negative in seven vials and unreadable in six cases because of cell toxicity or nonspecific fluorescence.

EIA results. Of the 45 culture-positive specimens, 25 (56.8%) were positive in the 2-h Adenoclone EIA. The average time to CPE for EIA-positive samples was 5.3 days (range, 2 to 14 days), whereas the average time to CPE detection for EIA-negative specimens was 15.5 days (range, 6 to 28 days). The EIA also had three nonreproducible positive results, but these were positive in only one of two duplicate wells and were associated with technical problems in the Behring ELISA Processor.

Monoclonal IFA test results. The Adenoclone monoclonal IFA test detected 39 (86.7%) of 45 culture-positive specimens in 72 h (Table 1). Of the six monoclonal-IFA-negative vials, two were scored as questionable. Fourteen samples could not be scored because most of the cell sheet was lost to toxicity or contamination. The average time to detection of CPE for the Adenoclone monoclonal IFA-positive samples was 7.4 days (range, 2 to 20 days) as compared with 16.2 days (range, 9 to 25 days) for the monoclonal IFA-negative vials.

Cell culture confirmation. A total of 114 cell culture isolates were tested by both Adenoclone monoclonal IFA test and EIA. The 35 serotypes of adenovirus from the American Type Culture Collection (adenovirus types 1 to 11, 13, 14, 19 to 37, and 39 to 41) were tested when CPE were detectable in culture. Sixty-three adenovirus cell culture isolates, 3 urine, 4 respiratory, 2 colon tissue, 20 stool, and 34 conjunctival specimens, were positive by all four of the methods used—culture, PDFA, monoclonal IFA test, and EIA.

None of the 16 heterologous viruses tested from American Type Culture Collection stock cultures or isolates from patients gave positive results with the Adenoclone monoclonal IFA test or EIA. These viruses included cytomegalovirus (CMV), HSV, varicella-zoster virus, respiratory syncytial virus, influenza viruses A and B, parainfluenza viruses 1, 2, and 3, rotavirus, and several enteroviruses, including echovirus types 6, 7, 9, 17 and coxsackievirus types A9 and B5.

Adenoviruses have been well recognized as significant viral pathogens in keratoconjunctivitis and respiratory infections (7, 9, 11, 13, 14, 17). However, the fastidious adenovirus serotypes 40 and 41 have only recently been described as causes of gastroenteritis (11). Requests are increasing for lab diagnosis of these enteric infections and chronic adenoviral infections in immunocompromised organ transplant recipients and acquired immunodeficiency syndrome patients. This growing recognition of adenovirus-associated disease and the development of antiviral agents such as ribavirin and interferon increase the need for rapid and accurate laboratory detection of adenoviruses (6, 13).

The use of culture and a polyclonal adenoviral antibody conjugate had three problems in the past. (i) The development of CPE in culture requires an average of 10 days (2 to 28 days), (ii) the fastidious gastroenteritis serotypes were not detectable in routine culture (1, 8, 11), and (iii) the polyclonal anti-adenovirus antibody (PDFA) gave positive results with CMV strain AD-169 (American Type Culture Collection) and several urine specimen isolates identified as CMV with monoclonal antibody to CMV nuclear antigen (Du Pont Biotech, Rockville, Md.). The pre-CPE vial culture method used with the polyclonal antibody detected 83% of culture-positive specimens in 5 days. Centrifugation has been reported to decrease detection time for several viruses (3, 4), but Darougar et al. reported equivalent results for adenovirus with centrifuged and uncentrifuged vials (2). In a busy clinical laboratory, many blood, body fluid, and neonatal specimens tested for HSV and CMV are set up for centrifugation enhancement. Therefore, we elected to evaluate the new adenovirus monoclonal antibody without the added expense of centrifugation.

The sensitivity of EIA and the monoclonal IFA test for adenovirus detection in direct specimens varied with the amount of virus present, as reflected by the time to development of CPE (Fig. 1). Results appeared to be affected by sample dilution, since virus was detected by direct EIA in only 14.3% of the culture-positive stool specimens diluted in PBS (1:10) and freezing medium (final dilution, 1:20), whereas 60% of the culture-positive specimens detected

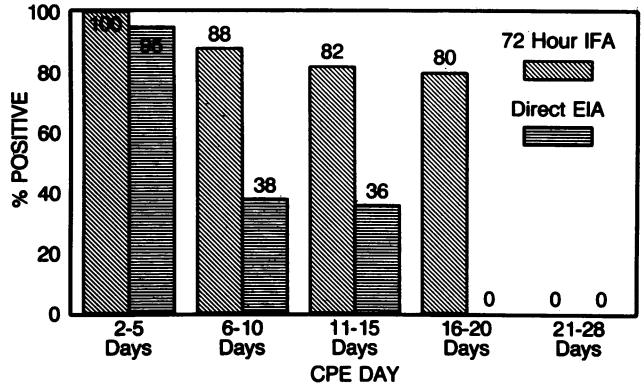

FIG. 1. Number of positive results detected by EIA and the IFA test compared with CPE.

among fresh stool specimens were diluted only in PBS (1:10). For all of the specimen sources, dilution in 3 ml of transport-antibiotic medium yielded an EIA sensitivity of 52.7%, compared with 66.6% sensitivity when only 2 ml of transport medium was used. Loss of sensitivity upon sample dilution in medium has previously been reported for EIA tests for HSV and *Chlamydia trachomatis* antigens (10, 12). The overall sensitivity of the Adenoclone EIA with direct specimens was 56.8%, which is in agreement with previous reports of 50 to 60% sensitivity for EIA detection of adenovirus antigen (9, 18).

The Adenoclone monoclonal IFA test performed at 72 h in shell vial cultures yielded the best combination of speed and sensitivity—86.7% of culture-positive specimens detected in 72 h.

The specimens with a higher concentration of virus, as reflected by a shorter interval for development of CPE, were those positive for adenovirus by EIA or the monoclonal IFA test. Of those specimens in which CPE were detected in 5 days or less, 100% and 95% were positive by the monoclonal IFA test and the EIA, respectively, but the EIA detected none of those which were CPE positive after day 15, and the monoclonal IFA test detected none which were CPE positive after day 19 (Fig. 1).

The specificity of the Adenoclone in all of the assays was found to be 100%. For culture confirmation, with 114 isolates, including all 35 adenovirus serotypes, both the Adenoclone EIA and monoclonal IFA test were 100% sensitive.

Among the advantages of using the Adenoclone assays is the ability to detect adenovirus serotypes 40 and 41, not routinely cultivatable strains which are associated with gastroenteritis (1, 8, 11). One advantage of the EIA method over the IFA test is the potential for automation to process large numbers of samples. Forty-five specimens can be tested for both rotavirus and adenovirus on a single microtiter plate in a 2-h run. In the EIA, no specimens are lost to toxicity, which is always a problem with stool specimens in cell culture. However, both the EIA and the IFA test can give false-positive results which may be associated with technical performance difficulties such as inadequate washing.

In summary, the Adenoclone monoclonal antibody tests were found to be sensitive and specific for adenovirus cell culture confirmation by EIA and the IFA test. The 72-h IFA test was also found to be a rapid and sensitive method for detection of adenovirus antigen from clinical specimens.

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