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Evaluation of a Commercial Monoclonal Antibody-Based Enzyme Immunoassay for Detection of Adenovirus Types 40 and 41 in Stool Specimens

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A commercial monoclonal antibody-based enzyme immunoassay (Adenoscreen; Mercia Diagnostics Ltd., Guildford, United Kingdom) for the detection of adenovirus types 40 and 41 in stool specimens was evaluated. Two assay modes were tested. In the first, 177 stool samples were screened for the presence of adenovirus type 40 or 41 (assay mode 1). Virus was detected in 79 of 82 specimens positive for adenovirus type 40 or 41 by a polyclonal antibody-based immune electron microscope test, giving a sensitivity of 96.3%. The enzyme immunoassay was negative in 91 of 95 stool samples which contained either other adenovirus serotypes or other viruses or were virus negative. The specificity was thus 95.8%. The positive and negative predictive values of this assay against immune electron microscopy were 95.2 and 96.8%, respectively, and the diagnostic accuracy was 96.0%. Viruses from the three false-negative enzyme immunoassay stool samples were verified as adenovirus type 40 or 41 by restriction enzyme analysis, monoclonal antibody-based immune electron microscopy, or both. Two of the three false-negative stool samples were subsequently concentrated by ultracentrifugation, and one of the two stool samples was then positive by enzyme immunoassay. The third false-negative virus was typed as adenovirus type 41 in the second (serotyping) enzyme immunoassay mode. The four enzyme immunoassay false-positive stool samples all contained other adenovirus serotypes (two were type 2, and two were type 5), but no cross-reactivity was seen with other strains of these serotypes and the results probably reflected simultaneous excretion of adenovirus type 40 or 41 with other adenovirus serotypes. In the second assay mode viruses from 15 stool samples were serotyped. The results by enzyme immunoassay (4 were type 40 and 11 were type 41) correlated completely with previous results from restriction endonuclease analyses. The commercial enzyme immunoassay system showed excellent sensitivity and specificity for the detection of adenovirus types 40 and 41 in stool specimens and will make an important contribution to the accurate diagnosis of adenovirus gastroenteritis.

In recent years adenovirus type 40 (Ad40) and Ad41 (2) have been shown to be causative agents of infantile gastroenteritis (1, 13), which is second in importance as a cause of gastroenteritis only to rotaviruses (10, 13). Ad40 and Ad41 belong to subgenus F of the adenovirus group and therefore are abbreviated to Ad-F (which, depending on context, means either both Ad40 and Ad41 or only one of the two serotypes) throughout this report. Ad-F can be detected in feces but so, also, can other adenovirus serotypes which have no proven role in diarrheal disease (14). It is essential, therefore, to serotype adenoviruses identified in stool specimens to make an accurate diagnosis of adenovirus gastroenteritis (15). Immunoassay techniques based on polyclonal antisera and suitable for diagnostic use (enzyme immunoassay [EIA] and immune electron microscopy [IEM]) have been developed (8, 9, 16) but are not widely available because of limited access to reagents. Recently, however, Ad-F-specific monoclonal antibodies have been developed (7, 11; J. C. de Jong, et al., 7th Int. Congr. Virol., Edmonton, Alberta, Canada, Abstr. R11.18, p. 111, 1987) which are effective in EIAs (6, 12) and IEM (D. J. Wood and J. C. de Jong, manuscript in preparation). In this report we describe an evaluation of the first commercial monoclonal antibody-based EIA for Ad-F viruses.

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MATERIALS AND METHODS

Stool specimens. A total of 177 virologically characterized stool samples (Table 1) from children in North Manchester, United Kingdom, with gastroenteritis were tested. All samples were stored at −40°C before use.

Electron microscopy and IEM. Stool samples were examined by negative stain electron microscopy after they were concentrated by ultracentrifugation. Viruses were identified by morphological criteria (3). Adenovirus-positive samples were also tested by IEM as described previously (16). Ad-Fs were identified by reaction with polyclonal sera reactive with Ad40 and Ad41 but not other adenovirus types (16).

Virus culture. All stool samples were cultured in primary monkey kidney, Vero, Hep-2, and human embryo fibroblast cells; and adenoviruses were identified by neutralization (16). Thirty-two samples were additionally cultured in Graham 293 cells (4).

Restriction enzyme analysis. Cell culture isolates from stools positive for Ad-F by IEM were typed as Ad40 or Ad41 by digestion with *Sma*I as described previously (16).

EIA. Stool samples were coded and tested by a commercial monoclonal antibody-based EIA (Adenoscreen; Mercia Diagnostics Ltd., Guildford, United Kingdom) by the instructions of the manufacturer. Briefly, a 10% (wt/vol) fecal

TABLE 1. Ad-F EIA results on virologically defined stool samples

Type of stool	No. of samples with the following EIA result:	
	Positive	Negative
Ad-F positive	77	3
Mixed Ad-F/non-Ad-F[a]	2	0
Non-Ad-F positive[b]	2	26
Other virus positive[c]	0	33
Virus negative[d]	0	32

[a] Two Ad-F/Ad2 mixtures
[b] A total of 6 Ad1, 5 Ad2, 2 Ad3, 2 Ad4, 3 Ad5, and 12 untyped adenoviruses.
[c] Nine rotaviruses, eight astroviruses, six small round-structured viruses, five caliciviruses, four enteroviruses, and one small round featureless virus.
[d] By electron microscopy and culture (including 293 cultures).

suspension was made in 0.1 M phosphate buffer (pH 7.2) with a vortex mixer. The suspension was allowed to stand for 10 min, and then 100 μl was added to microtiter wells coated with rabbit antiserum to adenovirus group antigen (raised against Ad40 strain Dugan) and incubated at 37°C for 1 h. Ad40 and Ad41 (positive controls) and extraction buffer (negative control) were included in each assay. Plates were washed five times with wash buffer, and then 100 μl of a biotinylated monoclonal antibody–peroxidase-labeled streptavidin mixture was added immediately to each well and incubated at 37°C for 1 h. The wash cycle was repeated, and 100 μl of diluted substrate (tetramethyl benzidine) was added and incubated at room temperature for 30 min. Results were read visually immediately on completion of substrate incubation. The reaction was then stopped by the addition of 2 M H_2SO_4, and the A_{450} was determined in a plate reader (Titertek Multiskan; Flow Laboratories Ltd., Irvine, Scotland) that was blanked on air. Two monoclonal antibodies, specific for Ad40 or Ad41, were provided in the kit and were mixed together for the detection of Ad40 or Ad41 (assay mode 1). All samples were tested in this way. Additionally, the monoclonal antibodies were used individually to serotype 15 Ad-F-positive stool samples characterized by restriction enzyme analysis (assay mode 2).

Analysis of data. EIA sensitivity was calculated by dividing the number of EIA-positive results by the number of IEM-positive results. The specificity was calculated by dividing the number of EIA-negative results by the number of IEM-negative results. The positive predictive value was obtained by dividing the number of stool samples that were positive in both IEM and EIA by the same value plus the number of stool samples that were positive by EIA and negative by IEM. The negative predictive value was calculated by dividing the number of stool samples that were negative in both IEM and EIA by the same value plus the number of stool samples that were negative by EIA and positive by IEM. Finally, the diagnostic accuracy was defined as the number of stool samples that were positive in both assays plus the number of stool samples that were negative in both assays divided by the total number of stool samples tested.

RESULTS

Ad-F EIA (assay mode 1). One hundred and seventy seven stool samples were tested for the presence of Ad-F (i.e., either Ad40 or Ad41) by EIA (Table 1). The assay was

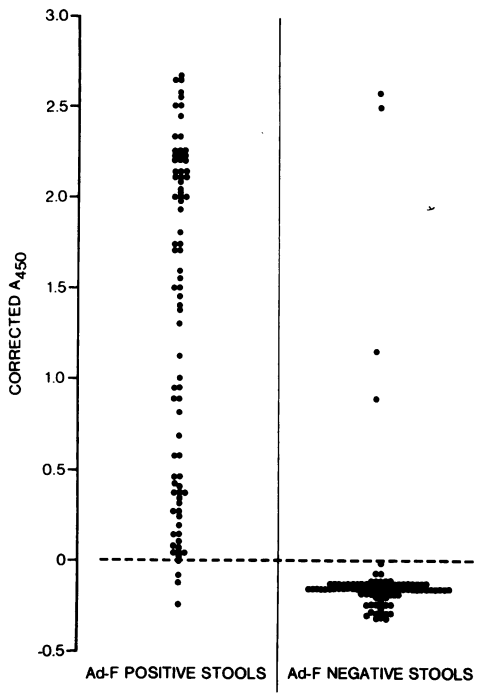

FIG. 1. Distribution of corrected A_{450} values for Ad-F-positive and -negative stool samples in Ad-F EIA. Corrected values were calculated by subtracting the cutoff value (negative control $A_{450} \times 3$) from the actual A_{450} reading. Negative values represent negative results.

positive for 79 of 82 stool samples positive for Ad-F by IEM and thus had a sensitivity of 96.3%. A wide scatter of A_{450} values was seen (Fig. 1). The assay was negative for 91 of 95 Ad-F IEM-negative stool samples, giving a specificity of 95.8%. The four EIA-positive, IEM-negative stool samples all gave repeatedly high A_{450} values (Fig. 1). The positive predictive value of the assay against IEM was 95.2% (79 of 83 stool samples), the negative predictive value was 96.8% (91 of 94 of stool samples), and the diagnostic accuracy was 96.0% (170 of 177 stool samples). Four samples were scored positive on visual inspection, but absorbance values were below the negative cutoff value (Table 2). The color was weak in all four instances, and the A_{450} ranged from 0.192 to 0.280. On repeat testing concordance between visual inspection and absorbance values was obtained with two samples that were positive and two that were negative. These results were used in the data analysis. However, all four samples were found to be Ad-F positive by IEM when the code was broken.

Analysis of false-negative results. Three samples were scored as falsely negative by Ad-F EIA, although two of

TABLE 2. Discrepancies between visual inspection and absorbance values in Ad-F EIA

Specimen no.	Results by eye		A_{450}			
			Original		Repeat	
	Original	Repeat	Specimen	Cutoff	Specimen	Cutoff
87/3564	+	−	0.197	0.300	0.162	0.279
87/3559	+	+	0.192	0.300	0.709	0.279
86/5950	+	−	0.280	0.351	0.091	0.279
87/0373	+	+	0.245	0.249	0.470	0.279

TABLE 3. Further tests on false-negative samples obtained by EIA

Specimen no.	Result when tested with:		
	Concentrated suspension	Diluted suspension	Single monoclonal antibody (Ad41)
87/3564	−	−	ND[a]
86/5950	+	−	ND
86/4881	ND	ND	+

[a] ND, Not done.

these three gave a weak color reaction when the assay was read by eye as described above. The three samples were investigated further (Table 3). One was found to be positive by EIA after concentration by ultracentrifugation, one was positive when it was reacted with the Ad41 monoclonal antibody alone (assay mode 2, see below), and one remained negative with both more concentrated and more dilute suspensions.

Analysis of false-positive results. Four samples were scored as falsely positive by Ad-F EIA, and all four contained other adenovirus serotypes (two were Ad2 and two were Ad5). With stool specimens the EIA results were repeatedly highly positive, whereas the Ad-F IEM was repeatedly negative. Ad2 or Ad5 was confirmed in cell culture isolates from each specimen by restriction enzyme analysis, neutralization, or both. Ad-F EIA was negative with three of four of these cell culture isolates, and the other isolate was not tested.

Serotype-specific EIA (assay mode 2). Fifteen Ad-F-positive stool samples from which virus was typed by restriction enzyme analysis were also tested in a serotype-specific EIA (i.e., with each monoclonal antibody alone). The results (Table 4) showed complete concordance. Furthermore, the Ad41 control A_{450} values were consistently higher with the Ad41 monoclonal antibody alone than in the Ad-F EIA, in which both monoclonal antibodies were mixed together (data not shown).

DISCUSSION

Accurate diagnosis of adenovirus gastroenteritis requires specific identification of Ad-F viruses in stool samples (15). Tests that detect adenoviruses but that do not discriminate Ad-F from non-Ad-F serotypes (5) are misleading. When Ad-F-specific tests have been used, it is apparent that these viruses are second only to rotaviruses as a cause of infantile gastroenteritis (10, 13). However, the limited availability of the diagnostic tests so far developed means that Ad-F infections are grossly underreported. The production of a commercially available test is therefore a major advance. We report here an evaluation of this kit. The kit allows identification of either Ad-F type (i.e., Ad40 or Ad41) or the individual serotype in a specimen by use of the monoclonal antibodies as a mixture or singly, respectively. We chose to concentrate on the Ad-F test (assay mode 1) for reasons of reagent economy, and also because a diagnosis of Ad-F infection is clinically as useful as identification of the serotype. For epidemiological purposes, the serotype assay (assay mode 2) would be more useful.

TABLE 4. Serotype-specific EIA

Type of stool	No. of EIA results	
	Ad40 positive	Ad41 positive
Ad40 positive	4	0
Ad41 positive	0	11

We found the test easy to perform, and the results showed a high degree of specificity (95.8%) and sensitivity (96.3%) compared with IEM. Four specimens gave a positive EIA result but were negative by IEM. IEM is known to give falsely negative results in samples with low concentrations of virus (16). These four stool samples also contained non-Ad-F serotypes (Table 1), but there was no evidence of cross-reaction in the Ad-F EIA with cell culture isolates of these strains or of other strains of the same non-Ad-F serotypes in stool samples. We therefore conclude that these four samples were mixed Ad-F–non-Ad-F infections that were falsely negative by IEM.

On the other hand, three samples were positive by IEM but were initially negative by EIA. Two of these were found to be EIA positive on further examination, while one remained negative (Table 3). This last result may represent an Ad-F strain that was nonreactive with the monoclonal antibodies because of antigenic variation (13a). The majority of Ad-F strains encountered, however, which included a range of Ad41 DNA variants (D. J. Wood and A. S. Bailey, manuscript in preparation), were detected by the monoclonal antibodies.

The results obtained in this study are comparable with those of previously reported monoclonal antibody-based Ad-F EIAs (6, 12) and suggest that this approach provides excellent sensitivity and specificity for the detection of Ad-F infections. The technique described here is suitable for diagnostic and epidemiological applications and, if used in conjunction with rotavirus-specific tests, will allow identification of the causative organism in 90 to 95% of cases of diagnosable childhood viral gastroenteritis (10, 13).

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