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Detection of Enteric Adenoviruses With Synthetic Oligonucleotide Probes

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The abilities of hybridization probes to detect all human adenovirus types and to identify enteric adenovirus types were evaluated. The efficiency of hybridization was compared to other tests currently in routine laboratory use on clinical specimens from young children with gastroenteritis. Probes were derived from various regions of the adenovirus types 2 and 41 genomes, and were evaluated by hybridization with a series of DNA quantities from 1 μg to 10 pg of one adenovirus type from each human subgenus, lambda phage, and HEp 2 cells. The sensitivity of hybridization with the HPII probe (92.7%), containing the conserved hexon gene, compared well with EM (54.6%), culture and neutralization (45.5%), and enzyme immunoassay (61.8%). The sensitivity of detection of enteric adenovirus isolates by the cloned *Bg*/II D fragment probe (92.9%) and by a synthetic probe (85.7%), manufactured from type-specific sequences of the Ad41 hexon gene were comparable to Ad40/Ad41 specific enzyme immunoassay (84.6%). Hybridization was found to be a sensitive method of adenovirus detection in comparison to traditional methods of laboratory diagnosis. Synthetic oligonucleotides enable specific detection of individual enteric adenovirus types. Hybridization had additional advantages over other tests in identifying cases of infection with more than one adenovirus type and in allowing an estimate of the concentration of adenovirus in the specimen.

KEY WORDS: hybridization, sensitivity, specificity, virus concentration

INTRODUCTION

Despite the specific detection of adenoviruses in 5–17% of young children with gastroenteritis [Christiensen, 1989], determination of the involvement of adenoviruses in the aetiology of gastroenteritis has been difficult. Adenoviruses have been found consistently in the stools of apparently healthy children during surveillance programs [Fox et al., 1977; Rodriguez et al., 1985]. The ubiquity of adenoviruses makes it difficult to establish criteria to define adenoviral agents of gastroenteritis and prevents unequivocal substantiation of adenoviral causation of diarrhoea [Madeley, 1983]. The problem is compounded by differences in pathogenicity and cultivability between adenovirus types. The readily grown, lower numbered adenovirus types can be carried asymptomatically [Fox et al., 1977; Kidd et al., 1982; Rodriguez et al., 1985], while isolates of the enteric adenovirus types 40 and 41 that cause the majority of clinical disease [Uhnoo et al., 1984; Wigand et al., 1983] have fastidious growth characteristics and tend to escape identification. Additionally, enteric adenoviruses present in dual infections are frequently not observed [Brown, 1985]. Adenoviruses of a different type or viruses of most other groups tend to overgrow enteric adenoviruses in culture, even when present at a lower concentration [Brown, 1990], and fastidious adenoviruses are underdiagnosed.

Adenoviruses are found as the only pathogen present in a high proportion of stools of sick children [Christiensen, 1989] and should be included in a diagnostic protocol for pediatric gastroenteritis. An adenovirus test should operate directly on the initial specimen, to avoid difficulties with culture. The capacity of the test to distinguish the enteric adenovirus types, which have a specific association with gastroenteritis, would be advantageous. In this study we examined cloned and synthesized sequences for the detection of all adenovirus types and for the specific identificationof enteric types by hybridization. The sensitivity and specificity of the hybridization probes were evaluated in comparison to other conventional methods of adenovirus detection on clinical specimens from children with gastroenteritis.

MATERIALS AND METHODS

Virus and Probe Preparation

Cell lines HEp 2, A549, and 293; and adenovirus types Ad31 strain 1315; Ad7 strain Gomen; Ad2 strain Adenoid 6; Ad8 strain Trim; Ad4 strain RI-67; Ad40

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strain Dugan, and Ad41 strain Tak from each subgenus A to F were obtained from American Type Culture Collection (ATCC, 12301 Parklawn Drive, Rockville, MD). Viruses were cultured, purified on CsCl density gradients, and extracted as previously described [Scott-Taylor and Hammond, 1992]. Fragments of the adenovirus genome Ad41 strain Tak were amplified in competent *E. coli* strain Jm109 in plasmid pGem 3Z (Promega Biotech, Mississauga, Ontario). *Eco*RI fragments A, B, and C were cloned in plasmids p41EAC (containing both A and C fragments), p41EA, p41EB, and p41EC. Ad41 hexon gene sequences were electroeluted from p41EA after digestion with *Sal*I *and Hin*dIII. An attempt was also made to isolate the type-specific hexon gene sequences by cloning *Hin*dII fragments of the p41EA plasmid in the blunt-ended *Sma*I site in the pGEM 3Z vector. Useful transformants were identified by colony hybridization with the *Sal*ID fragment of the Ad41 genome. The *Bgl*IID fragment probe was kindly donated by Howard Takiff in the form of an insert in vector pAT153 [Takiff et al., 1985]. The HPII probe, a *Hin*dIII-*Pvu*II fragment enclosing the complete sequence of the Ad2 hexon gene, was evaluated in previous experiments as containing the most conserved adenovirus sequences, which enable this probe to detect all adenovirus subgenera with uniformity [Scott-Taylor et al., 1992]. Adenovirus DNA sequences were analysed with Pustell Sequence Analysis software (IBI, New Haven, CT) on genomic sequences recorded by Genbank. The suitability of oligonucleotide sequences for use as probes was determined with the program Oligo (National Bioscience, Hamel, MN).

Treatment of Stool Specimens

Between July 1990 and June 1991, 1,071 stool specimens received at the Cadham Provincial Laboratory were examined for the presence of adenovirus by electron microscopy (EM), tissue culture, and enzyme immunoassay (EIA). Ten percent stool suspensions were made by emulsification of approximately 1 g/10 ml phosphate buffered saline (PBS) containing antibiotics in polypropylene tubes with glass beads over a Vortex mixer. Suspensions were clarified by centrifugation at 3,020 × g (5,000 rpm in a Sorval RT600 centrifuge) for 20 min. EM examination was enhanced by ultracentrifugation of clarified suspensions onto formvar-coated grids by means of an airfuge [Hammond et al., 1981]. Two commercial immunoassay kits were employed for detection of all adenoviruses and for identification of enteric types according to the manufacturer's instructions (Cambridge Bioscience, Worcester, MA). Culture was performed by inoculation of 100 μl of clarified suspension applied to semiconfluent monolayers of 293 cell, primary RMK and HEp 2 cell lines. Specimens which grew virus in the conventional HEp 2 or RMK cells were further tested with neutralizing antisera (obtained from ATCC) to the 6 lowest numbered species. Non-neutralized virus isolates were identified by restriction analysis. Two monoclonal antibodies specific to Ad41 or Ad40 [Wood et al., 1989], supplied by Jan de Jong, were used in a blocking immunoassay as a further means of identification of a sample of 5 uncultivable isolates. Identification was determined by the ability of the specific antisera to block the binding of the isolates to microtitre wells coated with a capture antibody (Ahluwalia et al., in preparation).

Spot Blot Methods and Hybridization

The specificity of probes was evaluated with dilutions of DNA of one type of adenovirus from each subgenus, lambda phage, and HEp 2 cells, spotted at 10 μg to 100 pg per ml. DNA preparations were denatured with the addition of 0.1 volume of 3 M NaOH, neutralized after 30 min incubation with 0.1 volume of 3 M ammonium acetate, and equilibrated to physiological salt conditions with the addition of 0.3 volumes of 20× SSC (1× SSC = 0.15 M NaCl/0.015 M Na citrate). Then 150 μl of each dilution was applied by a slot blot apparatus (Schleicher and Schuell, no. 03431, Keene, NH) under very low vacuum to nylon membrane prewetted in 6× SSC.

Preliminary investigation demonstrated that the protein extraction of the clarified stool suspension improved the clarity and completeness of hybridized spots, as noted by Kidd et al. [1982]. Stool suspensions in 450 μl aliquots were incubated with 50 μl of 10% SDS and 250 μg of proteinase K for 30 min at 37°C before extraction with phenol and chloroform. Extracted samples were then boiled and cooled in ice water before 150 μl of sample were applied to prewetted nylon membranes with the slot blot manifold under low vacuum. Membranes were washed twice in 6× SSC, air dried, and baked at 80°C for 2 h prior to hybridization. Hybridization was carried out at 68°C as previously described [Scott-Taylor et al., 1992a] with at least 10^7 cpm/ml random prime labelled probe (Boehringer Mannheim, kit no. 1004 760). The melting temperatures of some hybridizations were lowered by the addition of formamide to the hybridization solution, according to the estimate that 1% formamide lowers the melting temperature by 0.72°C [McConaughy et al., 1969].

RESULTS

Evaluation of Sequences of the Adenovirus Species 41 Genome for Specific Detection of Enteric Adenovirus

Ad41 *Eco*RI fragments A, B, and C, together comprising 84% of the genome [Scott-Taylor et al., 1992], were used to evaluate the specificity of Ad41 sequences for the detection of enteric adenoviruses. The *Eco*RI fragments, cloned in plasmid vectors, were hybridized with a series of adenovirus DNA preparations of each subgroup. The reaction of the DNA dilutions with a genomic Ad41 DNA probe is shown in the first panel of Figure 1 as the standard to which other probe reactions were compared. The reaction of plasmid p41EA with the subgroup DNAs is shown in panel ii. This large plasmid, containing over 50% of the Ad41 genome from 8 to 61 map units, reacted more strongly with the DNA

of other subgroups than the whole Ad41 genomic probe, detecting lower amounts of heterologous subgroup DNA relative to the quantity of Ad41 DNA detected in the same autoradiographic time interval. Various *Pvu*I fragments were electroeluted from the p41EA plasmid to assess the specificity of isolated central sequences of the Ad41 genome as probes. *Pvu*I fragments B, D, E, and F, extending from map units 26 to 48, 48 to 59, 17 to 21, and 21 to 26, respectively (Fig. 1, RE map), all demonstrate reactions (Fig. 1, panels iii, iv, v, and vi) comparable to the parent plasmid. Isolation of segments of the *Eco*RI A fragment by cleavage with *Pvu*I did not demarcate any area able to better distinguish between the DNA of Ad41 and other types. The *Pvu*I F fragment shown in panel vi may be useful as a subgenus F specific probe. This fragment detected Ad41 DNA within one log dilution of the reaction with Ad41 DNA and distinguished between other subgroups by at least 3 log dilutions. *Pvu*I F fragment probe has an approximately equal reactivity with enteric types and would not detect other adenovirus types unless present at 1,000 times the concentration of Ad41 virions.

Strategies to Isolate an Ad41 Type-Specific Probe

A number of fragments from different areas of the Ad41 genome were examined for their ability to differentiate between enteric adenovirus types. The reactions of these various probes are shown in a succession of panels in Figure 2 as tested against both enteric adenovirus DNA preparations on membrane spotted with DNA of species of each subgroup. In comparison to the whole Ad41 DNA probe in the uppermost panel, the *Eco*RI B and C fragments inserted in pGEM 3Z vector have a relatively insensitive reaction with the DNA of other subgroups (panels ii and iv), corresponding with their position at the nonconserved right-hand end of the genome. The *Bgl*II D fragment, derived from the portion of the *Eco*RI B fragment nearer the right terminus of the Ad41 genome, hybridized with greater relative intensity with homologous Ad41 DNA than the parent plasmid. The reaction of the *Bgl*II D fragment with Ad40 DNA in panel iii of Figure 2 is highly equivalent in sensitivity to the homologous DNA reaction. The difference in sensitivity for Ad41 and Ad40 DNA, apparently only 2 to 4 fold, is the least of any of the Ad41 fragments tested, and the *Bgl*II D fragment was the best prospect for use as an enteric adenovirus specific probe defined.

Further attempts to distinguish an Ad41 specific probe were made by testing small restriction fragments from within the hexon gene that code for the type-specific epitopes of the capsid [Roberts et al., 1986]. The reaction of electroeluted *Sal*I D and *Hin*dIII I fragment probes (Fig. 2, panels v and vi) do not adequately distinguish Ad41 DNA from DNA of other types for use as specific probes. The reaction of a cloned *Hin*dII hexon fragment probe, called plasmid p41HH in panel vii of Figure 2, varies with the different subgroup DNA preparations. No Ad41 specific probe was isolated by cloning or electroelution of Ad41 DNA fragments.

Ad41 Synthetic Probes

Published Ad41 sequences were compared to Ad2 and Ad40 genes to determine exact sequences unique to Ad41. The longest stretches of unique Ad41 sequence in the available sequences were found in the hexon gene. The L1 surface epitope sequence [Roberts et al., 1986] was divided into 4 sequences of variation of 30 base pairs or more that could serve as diagnostic probes. The most suitable sequence, however, was a fifth unique stretch of 84 nucleotides from residues 1225 to 1308 of the Ad41 hexon sequence [Toogood and Hay, 1988] forming the L2 epitopic loop. This sequence was synthesized as two 40 base oligomers, designated Hex5A and Hex5B, signifying the fifth unique hexon region, with the following sequences:

Hex5A: GCAGCTACAGACACGTACTCTGGCATA-AAGGCCAATGGCC

Hex5B: AACCTGGACTGCAGACGACAATTATGC-CGACAGAGGGGCA.

The sensitivity of the two probes was tested empirically by hybridization with dilutions of adenovirus, λ phage, and HEp 2 DNA in conditions of increasing stringency as shown in a series of panels in Figure 3. Hex5A has a greater content of guanine and cytosine residues and could be used in conditions of greater stringency (Fig. 3A). Hex5B sequence is unique to Ad41. This is reflected in the loss of reactivity with Ad40 and nonhomologous DNA in reactions carried out above 35°C (Fig. 3B). The Hex5B probe was used in evaluation of the hybridization test with clinical samples.

Diagnosis of Adenovirus in Clinical Samples by Various Tests

Electron microscopy, EIA, and viral culture of stool specimens were performed routinely through the period of study. Adenovirus was detected by at least one conventional test in 55 specimens of the 1,071 stool samples examined. Isolates were identified by neutralization or restriction analysis. All identified enteric isolates had restriction patterns of the Ad41A strain [Scott-Taylor et al., 1990]. The positive samples were spotted in random order among 200 samples on a nylon membrane for hybridization. Suspensions used in prior evaluations of 9 adenovirus positive specimens were spotted to determine whether virus in original suspensions had deteriorated. Samples 81 and 88 were from a single specimen, spotted twice to ensure reproducibility. The 200 spotted samples consisted, therefore, of 55 unique adenovirus positive specimens, 28 of which were assessed as enteric isolates, 10 duplicates, and 135 adenovirus negative specimens. Twelve of the adenovirus negative samples contained rotavirus, 8 grew enteroviruses, and small round virus particles were seen by electron microscopy in 6 and coronavirus in 1 more. The specimens reacting with the various diagnostic

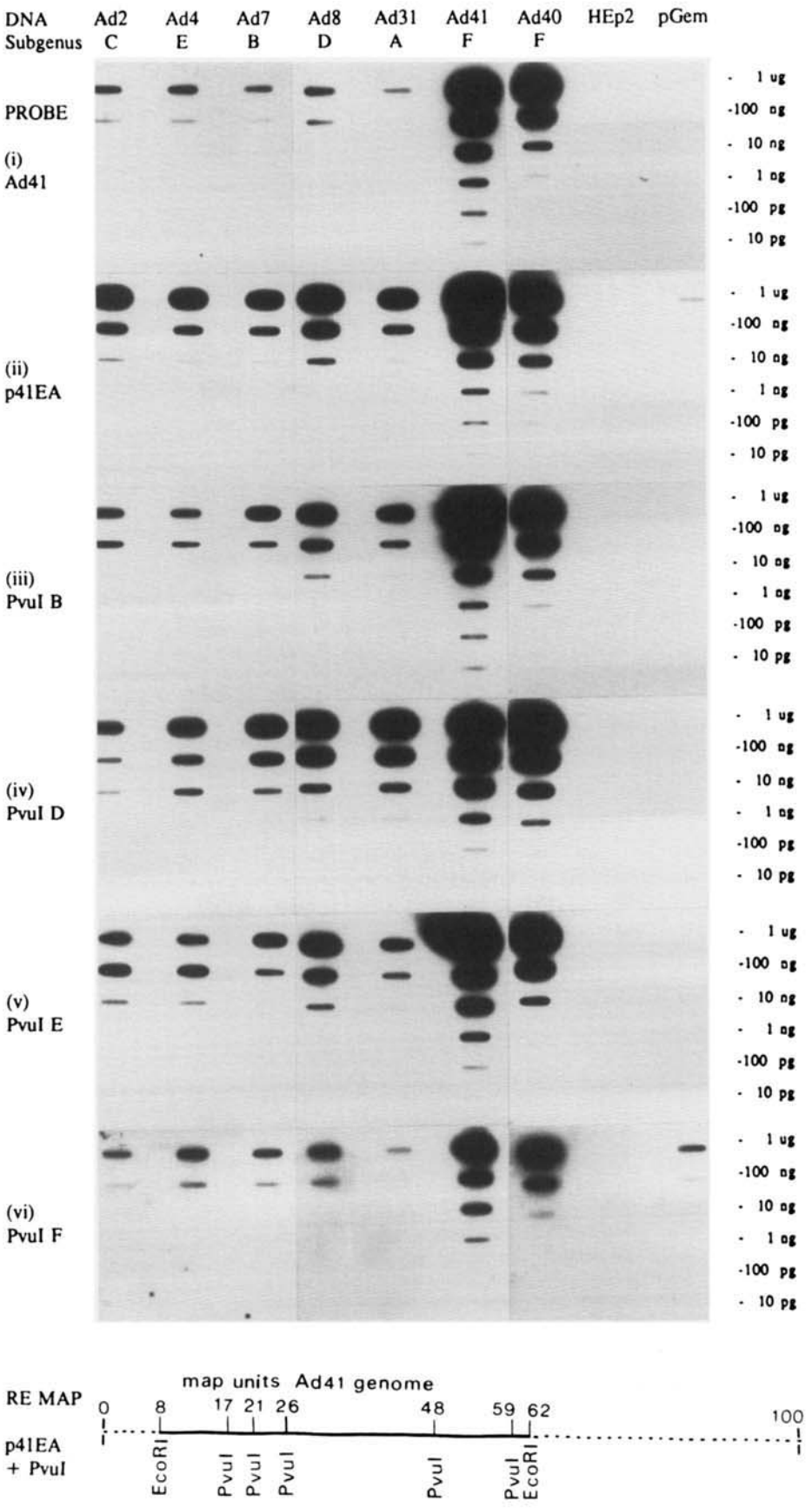

Fig. 1. Adenovirus subgenera DNA hybridized with plasmid p41EA and electroeluted *Pvu*I fragments. Then 100 μl aliquots of λ phage, cellular and adenovirus DNA of one species from each human subgenus, spotted in tenfold dilutions, were hybridized at 68°C with genomic Ad41 DNA **(i)**, plasmid p41EA **(ii)**, and the electroeluted *Pvu*I fragments B **(iii)**, *Pvu*I D **(iv)**, *Pvu*I E **(v)**, and *Pvu*I fragment F **(vi)**. The *Pvu*I map of the p41EA plasmid is shown at the **bottom.** Autoradiographs were developed after 48 hr.

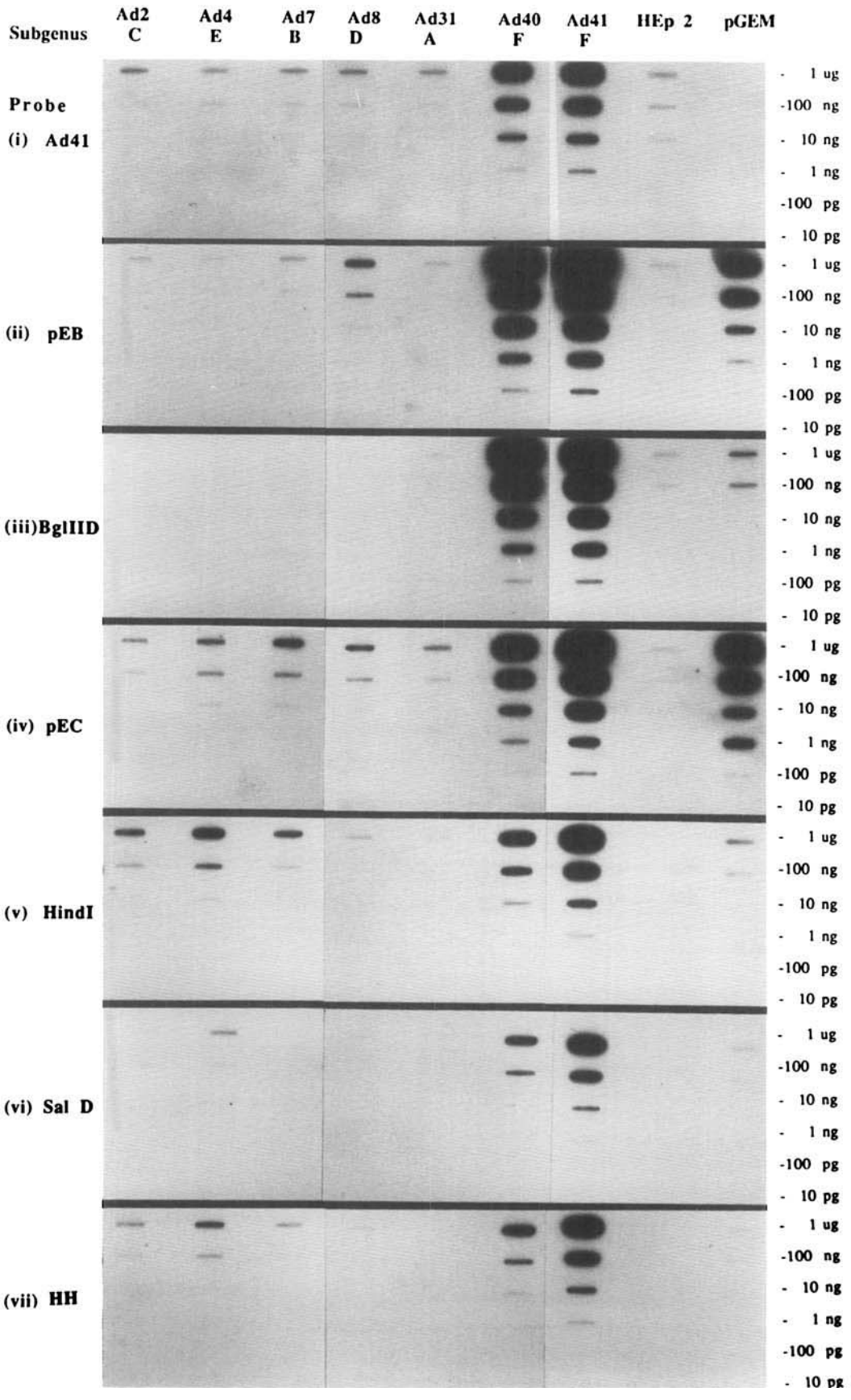

Fig. 2. Hybridization of Ad41 *Eco*RI and hexon gene fragments with the DNA of each subgenera. Log dilutions of DNA of Ad40 and one species from each adenovirus subgenus were hybridized at 68°C with genomic Ad41 DNA **(i)**, *Eco*RI fragment B plasmid p41EB **(ii)**; *Bgl*II D fragment containing plasmid **(iii)**; *Eco*RI C fragment containing plasmid p41EC **(iv)**; fragment *Sal*I D **(v)**; fragment *Hin*dIII I **(vi)**; and a *Hin*dII hexon gene fragment containing plasmid **(vii)**.

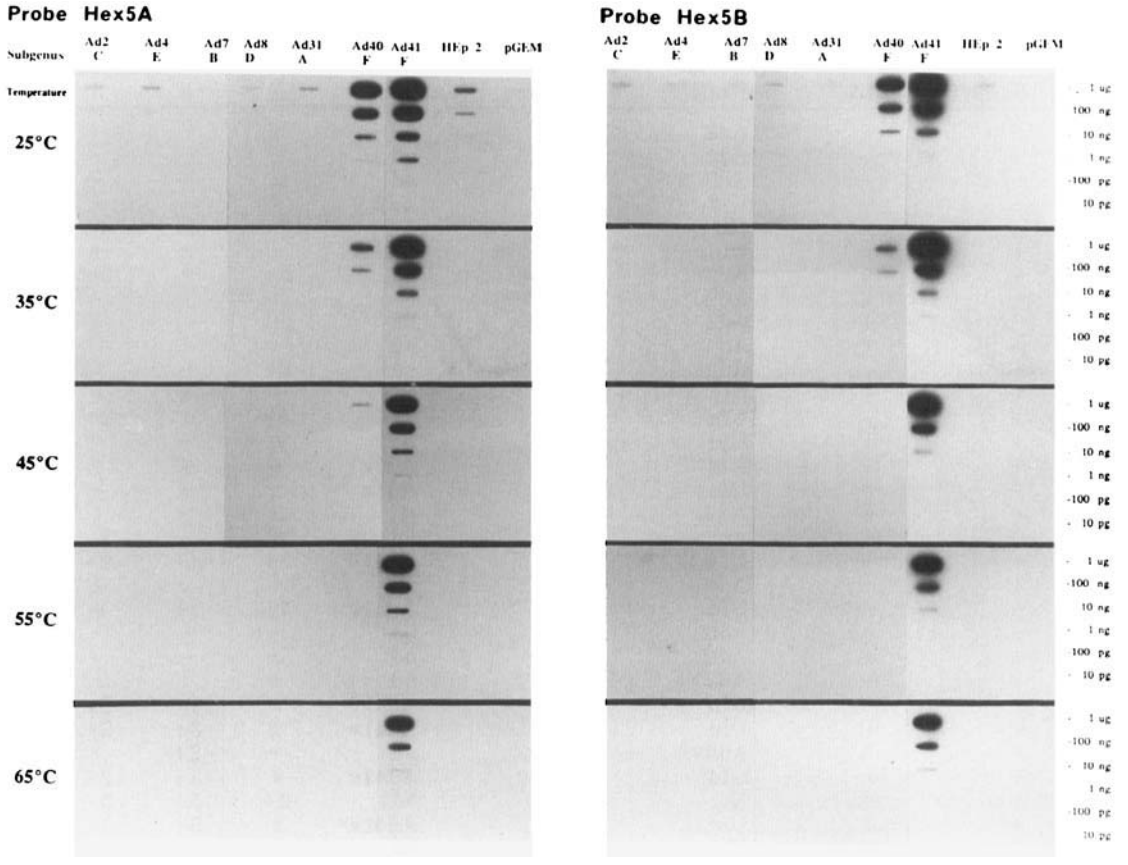

Fig. 3. Hybridization of the Hex5A and Hex5B probes with subgenera DNA at various stringencies. The synthetic probes were hybridized with membranes at 25°C, 35°C, and 55°C with either 0% or 14% formamide in the hybridization solution. Each membrane was enclosed with film for 48 hr.

tests are compiled in Table I in the order spotted on the hybridization membrane. A total of 60 of the samples, including 51 of the unique specimens, reacted with the HPII probe in the first panel of Figure 4. The *Bgl*II D fragment and synthetic Hex5B probes in the two panels below reacted with 26 and 24 specimens, respectively. The amount of viral DNA in the specimen could be estimated from the dilution series of control DNA below the specimens. The Ad41 genomic DNA probe and plasmid p41EC, containing the Ad41 *Eco*RI C fragment, hybridized with specimens containing adenoviruses other than enteric types (reactions not shown). Cloned probes p41EC and *Bgl*II D reacted with negative specimens 122, 124, and 166. Specimens 27 and 199 reacted strongly with all Ad41 DNA probes, although types Ad2 and Ad5 emerged from culture. These specimens were subsequently tested with the subgenus F-specific EIA and were found to harbour a conventional type as well as an enteric adenovirus in concurrent infection. A sample of 5 of the 9 uncultivable specimens not identified to type was tested in the blocking assay. Preincubation with the Ad41 monoclonal antibody reduced binding to the capture antibody by between 12% and 37%. The Ad40 antibody had no effect and for the purposes of calculation of test performance all the uncultivable specimens were presumed to contain Ad41. The sensitivity and specificity of the diagnostic tests are compared in Table II. The 92.7% sensitivity of the HPII probe in hybridization, detecting 51 of 55 unique specimens, compares favourably with conventional diagnostic tests. The specificities of the genomic Ad41 DNA, p41EC, and *Bgl*II D probes were evaluated on the detection of enteric adenovirus types and were reduced by reactions with unrelated adenovirus types or negative specimens. The synthetic DNA probe specificity was evaluated for Ad41 specimens alone. No false positives were attributed to HPII or Hex5B probes. The predictive values of the tests (Table II) indicate that most hybridization probes had greater reliability in reporting a positive or negative test result than the conventional diagnostic methods.

DISCUSSION

Hybridization demonstrated a higher sensitivity than the methods of adenovirus detection currently employed. Results indicate that hybridization could improve the efficiency of diagnosis of adenovirus infection in gastroenteritis by one and a half times or more over individual methods in routine use. The technique has great flexibility and can utilize cloned or synthetic sequences to diagnose groups or individual types of virus. Oligomeric probes can evidently form effective means

TABLE I. Detection of Adenovirus in 200 Clinical Samples by Various Tests*

Sample number	Electron microscopy	Group EIA	Culture neutraliz.	Enteric EIA	Restriction analysis	Hybridization probe 1	2	3	4	5
2	–	–	Ad2	nd	nd	±	–	–	–	–
10	–	–	Ad4	nd	nd	–	–	–	–	–
19	–	–	Ad5	nd	nd	±	–	–	–	–
20[A]	–	–	UId	–	Ad2	+	–	–	–	–
21	–	+	NG	+	NG	3+	2+	4+	3+	–
22	+	+	UId	+	Ad41v	±	±	+	±	+
27	+	+	Ad2	+	nd	5+	±	2+	2+	+
28	–	+	Ad2	nd	nd	+	–	–	–	–
34	–	–	Ad2	nd	nd	+	–	–	–	–
39	+	+	Ad4	nd	nd	+	±	–	–	–
43[A]	–	–	Ad2	–	nd	2+	–	–	–	–
44	+	+	NG	+	NG	+	±	–	+	+
45	+	+	UId	+	Ad41v	4+	+	2+	+	+
51	+	+	UId	nd	Ad2v	+	–	–	–	–
53	+	+	UId	–	Ad3v	+	+	–	–	–
57	–	–	UId	–	Ad1v	±	–	–	–	–
61	–	+	Ad2	nd	nd	±	–	–	–	–
63	–	–	Ad5	nd	nd	±	–	–	–	–
68	+	+	UId	+	Ad41v	5+	4+	2+	3+	4+
72[B]	+	+	Ad4	nd	nd	+	±	+	–	–
73	+	+	UId	+	Ad41v	4+	3+	+	2+	2+
77	–	+	Ad6	nd	nd	+	–	–	–	–
76	+	+	Ad2	nd	nd	±	–	–	–	–
78	+	–	Ad2	nd	nd	+	±	±	–	–
81[C]	+	+	UId	+	Ad41v	+	3+	2+	+	+
86	+	+	UId	+	Ad41v	±	3+	2+	+	+
87[D]	+	–	Ad5	nd	nd	+	2+	+	–	–
88[C]	+	+	UId	+	Ad41v	+	3+	2+	+	+
91[E]	+	+	NG	+	NG	2+	5+	5+	4+	3+
94	+	+	UId	+	Ad41v	+	5+	3+	2+	2+
95[E]	+	+	NG	+	NG	2+	5+	4+	3+	3+
97[F]	–	+	Ad3	nd	nd	+	–	–	–	–
104[G]	–	+	Ad2	nd	nd	+	–	±	–	–
107	–	+	UId	–	Ad2v	+	–	–	–	–
108[B]	+	+	Ad4	nd	nd	±	±	±	–	–
112	+	+	UId	+	Ad41v	2+	+	2+	+	3+
116	+	+	UId	+	Ad41v	4+	2+	5+	5+	5+
119	–	+	UId	+	Ad41v	±	–	+	+	±
122	–	–	NG	nd	nd	–	–	±	±	–
124	–	–	NG	nd	nd	–	–	±	±	–
128	+	+	UId	+	Ad41v	+	+	±	+	+
129	–	+	Ad2	nd	nd	2+	–	±	–	–
133[H]	–	+	NG	+	NG	±	–	–	–	±
134[G]	–	+	Ad2	nd	nd	+	–	+	–	–
137[D]	+	–	Ad5	nd	nd	+	+	–	–	–
139	+	+	UId	nd	Ad12	±	±	–	–	–
141	–	+	NG	+	NG	3+	4+	2+	2+	3+
142	+	+	UId	+	Ad41v	2+	2+	+	+	2+
155	–	–	Ad1	nd	nd	–	–	–	–	–
159	+	–	NG	–	NG	–	–	–	±	–
160	+	+	NG	+	NG	2+	2+	–	2+	2+
164[I]	+	+	UId	+	Ad41v	2+	+	–	2+	2+
166	–	–	NG	nd	nd	–	–	±	–	–
167	+	–	NG	–	NG	±	–	–	±	–
169	+	–	UId	–	Ad41v	±	±	–	+	–
170[H]	–	+	NG	+	NG	±	–	±	–	±
175	–	+	NG	–	NG	–	±	–	+	–
176	–	–	Ad2	nd	nd	3+	–	–	–	–
182[B]	+	+	Ad4	nd	nd	±	±	±	–	–
184[I]	+	+	UId	+	Ad41v	2+	2+	–	+	±
186	–	–	Ad2	nd	nd	+	–	–	–	–
187	–	–	Ad1	nd	nd	2+	–	–	–	–
191[F]	–	+	Ad3	nd	nd	+	–	–	–	–
193	+	+	Ad2	nd	nd	+	+	–	–	–
195	–	–	Ad2	nd	nd	±	–	–	–	–
199	+	+	Ad5	+	nd	3+	2+	±	+	+
200	+	+	UId	+	Ad41v	5+	4+	+	2+	2+

*A to I = duplicate samples from same patient/stool; NG = no growth; UId = unidentified; nd = not done; v = variant strain. Hybridization probe 1 = HPII, 2 = Ad41, 3 = p41EC, 4 = *Bgl* II D, 5 = Hex5B.

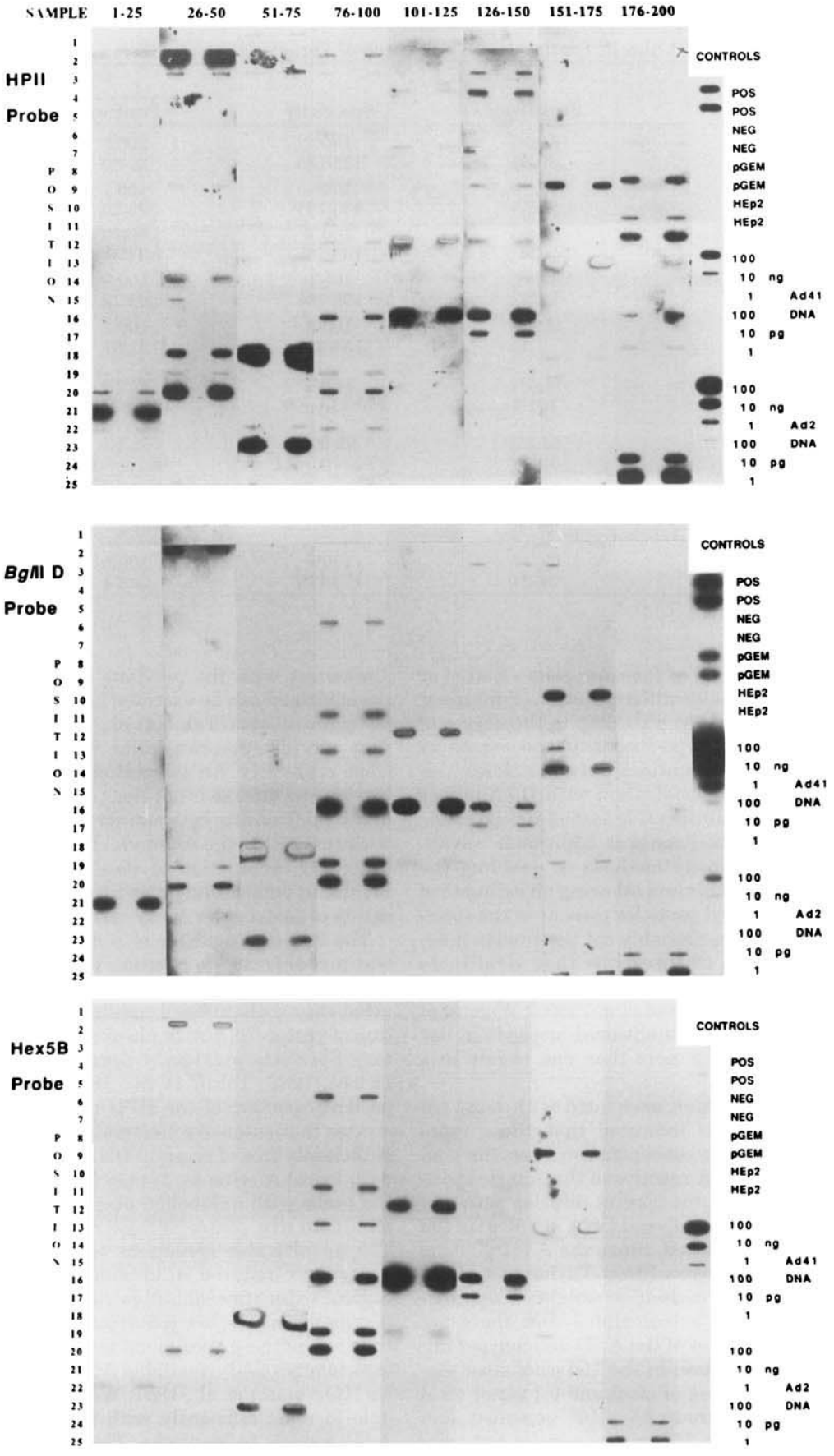

Fig. 4. Hybridization of 200 clinical samples with an HPII fragment probe I (**upper panel**); *Bgl*II D fragment probe (**middle panel**), and Hex5B synthetic probe (**lower panel**). The 10% stool suspensions, deproteinated and boiled, were spotted on membrane, baked, and hybridized with the probes at 68°C. The film enclosed with the membranes were developed for 48–60 hr.

TABLE II. Sensitivity and Specificity of Various Diagnostic Tests

Diagnostic method		Sensitivity	Specificity	Predictive value	
				Positive	Negative
Electron microscopy		54.6% 30/55	100% 135/135	100% 30/30	84.4% 135/135 + 25
Culture & neutralization		45.5% 25/55	100% 135/135	100% 25/25	81.8% 135/135 + 30
Group enzyme immunoassay		61.8% 34	100% 135/135	100% 34/34	87.1% 135/135 + 21
Enteric enzyme immunoassay		84.6% 22/22	100% 135/135	100% 22/22	97.1% 135/135 + 4
HYBRIDIZATION	HPII probe	92.7% 11	100% 135/135	100% 51/51	97.1% 135/135 + 4
	Ad41 probe	85.7% 101.4	94.9% 170/170 + 9	72.7% 20/24 + 9	97.7% 170/170 + 4
	p41EC probe	64.3% 18/28	93.9% 170/170 + 11	62.1% 18/18 + 11	94.4% 170/170 + 10
	*Bgl*II D probe	92.9% 26/28	98.8% 170/170 + 2	92.9% 26/26 + 2	98.8% 170/170 + 2
	Hex5B probe	85.7% 24/24	100% 170/170	100% 24/24	97.7% 170/170 + 4

of diagnosis, and selection of the appropriate shared or unique sequence can enable differentiation of groups or individual adenovirus types according to the degree of specificity required. The type-specific hexon sequences provide a means to differentiate between closely related adenoviruses by hybridization with DNA probes or neutralisation with antipeptide sera [Toogood et al., 1992]. Hybridization had several additional advantages over other diagnostic methods in enabling the detection of dual infections and allowing an estimate of the concentration of viral particles present in the specimen. Dual infections are probably not uncommon judging from the numbers of specimens that yield more than one adenovirus type upon careful culture [Brandt et al., 1986; Brown, 1985; Kidd et al., 1982; Wigand et al., 1983], and none of the traditional methods in use are capable of identifying more than one isolate in a specimen.

The intense hybridization associated with most enteric adenovirus isolates indicated that these types were excreted in greater concentration than the conventional types. The most reactive of the enteric specimens were defined from the control dilution series as present in excess of 100 ng of viral DNA in 150 μl of the 10% stool suspension spotted. Since the Ad41 genome comprises 34,600 base pairs [Scott-Taylor and Hammond, 1992] and has a molecular weight of approximately 23×10^6, it can be calculated that there are about 2.5×10^{10} molecules of the Ad41 genome per microgram of DNA. Therefore, in the 150 μl of stool suspension, containing 15 mg of stool and 0.1 μg of viral DNA, there are more than 2.5×10^6 genomes, and there are more than 1.7×10^{11} virus particles per gram in the most reactive stool specimens. This is in close agreement with the previous evaluation that enteric adenoviruses can be excreted in excess of 10^{11} particles per gram of stool [Takiff et al., 1981] and demonstrates that hybridization can define viral concentration with some reliability. An association between virus concentration and disease is not clear at present. Examination of the relationship between viral burden and prognosis with this technique could yield significant results. Additionally, evaluation of viral concentration may be helpful in determining a critical level for defining causation of gastroenteritis by certain adenovirus types.

The loss of specificity of some hybridization probes was probably due to reaction with plasmid DNA from bacterial flora, as several false-positive specimens reacted only with probes amplified in bacteria. The reaction of vector DNA with plasmids derived from alimentary flora has previously been observed [Huang and Deibel, 1988; Takiff et al., 1985]. The lack of false-positive reaction of the HPII probe, however, demonstrates that extensive electroelution can render probes sufficiently free of plasmid DNA contamination for use with faecal specimens. It may be advisable to saturate the probe with unlabelled plasmid DNA to completely eliminate this source of false results in hybridization. The uncultivable specimens tested with the blocking assay were reduced in binding by less than the 50% critical value that identifies an adenovirus type by the test methods. The low reduction in binding may signify that either these specimens were grossly denatured or that commercially available Ad41 monoclonal antibodies [Herrmann et al., 1987; Wood et al., 1989] are not able to react efficiently with local variant strains of Ad41 specific to Manitoba. The prevalent Ad41 strain in Manitoba was not detected by the first commercial

enzyme immunoassay [Scott-Taylor et al., 1990] marketed with monoclonal antibodies developed to the prototype strain Tak of Ad41 [Herrmann et al., 1987]. These observations suggest that DNA hybridization tests, less affected by the variation found in circulating strains of adenovirus types, may have more long-term efficacy than highly specific serological detection methods.

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