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Rapid Subgenus Identification of Human Adenovirus Isolates by a General PCR

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In most clinical situations involving adenovirus infection, subgenus (subgroup) identification of an adenovirus isolate is as informative as a finer identification by serotype. A PCR method which allows the identification of human adenovirus isolates as members of subgenera A, B:1, B:2, C, D, E, or F is described. It is based on a simple (nonnested) PCR using primers which bind to regions immediately flanking the VA RNA-encoding regions of human adenovirus genomes. The PCR allows amplification of DNA from all 49 human adenovirus prototype strains so far described. Since there are differences in the lengths of the VA RNA-encoding regions in adenoviruses of different subgenera, it is possible to differentiate some subgenera according to the size of the PCR product determined by electrophoresis. This forms the basis of an initial broad categorization of isolates as belonging to either (i) subgenus B:1, C, D, or E or (ii) subgenus A, B:2, or F. Subgenus identification is completed by a one-step restriction enzyme digestion and gel electrophoresis. The method was assessed by blind subgenus identification of 200 miscellaneous primate adenovirus isolates prepared by the reference laboratory at Bilthoven, The Netherlands. Identification at the subgenus level by PCR correlated 91.5% with the results of serotyping. A further 5.5% of isolates were correctly identified as belonging to one of two specified subgenera. Six of the 200 identifications (3%) were unsuccessful for various reasons, including weak PCR products, intermediate strains, and mistaken primate host. The method should serve as a rapid means of confirming adenovirus cytopathic effects in laboratories performing virus culture, with simultaneous subgenus identification of the isolate. It will also have relevance as an aid to conventional serotyping for epidemiological purposes, since for all adenoviruses except those belonging to subgenus D, neutralization tests need only involve a maximum of four type-specific antisera.

While the delineation of individual adenovirus serotypes is based on classical neutralization tests, human adenoviruses have been classified more broadly into six subgenera, A to F, on the basis of several biophysical and biochemical criteria (38). Adenoviruses of subgenus A are isolated almost exclusively from the gastrointestinal tract (2, 32), and the enteric adenoviruses of subgenus F are generally regarded as the second most important cause of infantile viral gastroenteritis in developed countries (27, 36) (Table 1). Subgenus B adenoviruses have been divided into two clusters, based largely on DNA analysis. Members of cluster B:1 are generally associated with acute respiratory infections, whereas the majority of cluster B:2 members have been isolated from the urinary tract (38). Subgenus C adenoviruses are associated with respiratory infections in early childhood, and shedding in feces may persist for many months (8, 12, 21). Some 31 serotypes constitute adenovirus subgenus D, and several of these have been significantly associated with eye infections (17). The newest members of subgenus D were isolated from the feces of AIDS patients (18, 33). Subgenus E has one member, adenovirus type 4 (Ad4), which can cause either respiratory or eye infection. It has been noted that a variant of Ad4 (Ad4a), which is genetically quite distinct from the prototype virus but has the same antigenic determinants, has been associated with outbreaks of eye disease (38).

The general detection of adenoviruses in clinical specimens can be achieved through conventional culture in epitheloid cell lines, immunofluorescence, radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), DNA hybridization, or, most recently, PCR (4, 15). More specific identification of adenoviruses usually relies, after initial detection by one or more of the above adenovirus genus-specific tests, on cell culture-based type-specific neutralization (14), restriction enzyme analysis of DNA extracted from infected cells (40), or, more rarely nowadays, hemagglutination inhibition (14). Alternatively, testing for adenoviruses can be based wholly on reagents for the specific detection of a single subgenus or individual types within a subgenus, for example, detection of subgenus F adenoviruses by ELISA (10, 13, 19, 35). While neutralization tests and DNA extraction can be time-consuming and depend on a sufficient yield of infectious virus, detection using reagents for one specific adenovirus type or subgenus, while effective, could mask the presence of dual adenovirus infections.

One PCR method using genus-specific primers (4) was extended to specifically differentiate subgenus F adenoviruses from those of other subgenera (5). In this paper, we describe a nonnested PCR method which allows rapid identification of all human adenoviruses at the subgenus level. The method is intended primarily for characterization of adenoviruses in cell culture fluids.

MATERIALS AND METHODS

Prototype adenovirus strains. All prototype strains except Ad16, Ad40, Ad41, Ad48, and Ad49 were obtained from the American Type Culture Collection. Prototype Ad16 was a gift from R. Wigand, Homburg, Germany. Ad40 reference

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TABLE 1. Conventional classification of the 49 human adenovirus serotypes into subgenera[a]

Subgenus and cluster	Adenovirus type(s)	Main tropism(s)[b]
A	12, 18, 31	Gut (S?)
B:1	3, 7, 16, 21	Respiratory tract (S); eye (S); gut (S?)
B:2	11, 14, 34, 35	Urogenital system (S?) (R); respiratory tract (S)
C	1, 2, 5, 6	Respiratory tract (S); gut (S?) (L) (H)
D	8–10, 13, 15, 17, 19, 20, 22–30, 32, 33, 36–39, 42–49	Eye (S); gut (S?) (P)
E	4	Respiratory tract (S); eye (S)
F	40, 41	Gut (S)

[a] Adapted from Wadell (39).
[b] S, symptomatic infection; R, reactivation in immunosuppressed patients; H, hepatitis in immunosuppressed patients; L, latent infection; P, particular serotypes from AIDS patients.

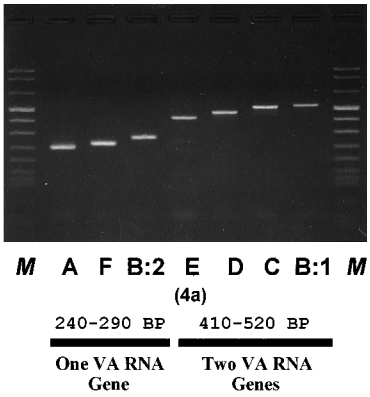

FIG. 1. VA RNA gene region PCR products derived from representative adenoviruses of the indicated subgenera, showing the two size ranges (240 to 290 and 410 to 520 bp). Approximate product sizes: subgenus A, 240 bp; F, 260 bp; B:2, 290 bp; subgenus E genome type Ad4a, 410 bp; D, 460 bp; C, 500 bp; and B:1, 520 bp. Lanes *M*, molecular size markers (Boehringer marker VIII) (1, 114, 900, 692, 501, 489, 404, 320, 242, 190, 147, 124, 110, 67, and 37 bp).

strain Hovi-X and Ad41 prototype strain Tak were originally characterized at Bilthoven, The Netherlands (11). Candidate adenoviruses Ad48 and Ad49 (33) were kindly donated by D. Schnurr, Viral and Rickettsial Disease Laboratory, Berkeley, Calif. Genomic variants Ad4a (28, 38) and Ad11a (29) were kindly donated by G. Wadell, Umeå, Sweden. All strains except prototypes Ad16, Ad40, and Ad41 were tested as received at the Lund laboratory without being passaged further.

PCR. The method used for PCR was largely as described elsewhere (4, 22, 30) but with several modifications. All manipulations were performed using good PCR laboratory practice to avoid cross-contamination and carryover of adenovirus DNA and PCR products (25, 26).

The reagent mixture for PCR was prepared in bulk prior to being dispensed in 49-μl amounts into 0.5-ml Geneamp reaction tubes (Perkin-Elmer Cetus, Norwalk, Conn.). The mixture contained 67 mM Tris (pH 8.8), 6.7 mM $MgCl_2$, 16.6 mM ammonium sulfate, 10 mM 2-mercaptoethanol, 6.7 μM Na_2EDTA, 100 μg of bovine serum albumin per ml, all four deoxynucleotides at 1.1 mM each, 80 pmol of each primer (VA3a, VA3b, and VA6; see below), and 1 U of *Thermus aquaticus* polymerase (Amplitaq; Perkin-Elmer). Additionally, the mixture contained 0.1% Triton X-100 to destabilize virus particles during the initial heating. One microliter of cell culture fluid from cultures showing typical adenovirus cytopathic effect was added to each reaction tube. It should be noted that in several cases a stronger PCR product was achieved if the culture fluid containing adenovirus was diluted 1/10 in sterile, distilled water prior to testing. The reason for this effect is not known. Negative-control tubes received either culture fluids from uninfected cultures or sterile, double-distilled water.

The reaction tubes were placed in a Perkin-Elmer model 480 thermal cycler and held at 94°C for 1 min. This was immediately followed by 35 cycles of 94°C for 1 min, 45°C for 1 min, and 72°C for 3 min. The final cycle had a prolonged extension time of 8 min.

Primers. PCR products from amplification of the VA RNA regions of several adenovirus serotypes were sequenced by using previously described primers (24). These sequences were then used to identify adenovirus genus-specific primer binding sequences situated immediately upstream and downstream of each VA RNA gene region (23). Upstream primer VA3a [5′-CGG T[G/C]A GGC G[T/C]G CGC AGT C-3′] has alternative nucleotides at two base positions, thus giving four different primer species. Downstream primer VA6 [5′-CGC AGC AC[C/G/T/A] GGA TGC ATC T-3′], has full degeneracy at one base position, thus giving four primer species. The detection of some adenoviruses, notably of subgenus F, was further improved by the addition of another VA3 primer species, designated VA3b (5′-CGG TAA GAC GGG CGC AAT C3′), which was synthesized separately and mixed with the VA3a primer preparation in equal proportions. VA3a and VA3b derive from the same upstream target sequence. All primers were synthesized by Scandinavian Gene Synthesis AB, Köping, Sweden, and all were 19 bases long.

Gel analysis of PCR products. All PCR products were electrophoresed in 2% agarose (type I-A; Sigma Chemical Co., St. Louis, Mo.) in Tris-borate-EDTA buffer at 10 V/cm for 75 min. The gels contained 0.1 μg of ethidium bromide per ml. Typically, 10 μl of a 50-μl PCR product mixture was loaded after being mixed with 3 μl of a loading dye containing 20 mM Tris-HCl (pH 8.0), 15% (wt/vol) Ficoll (Sigma), 20 mM Na_2EDTA, and 0.1 mg of xylene cyanol per ml. Molecular weight standards VI and VIII (Boehringer GmbH, Mannheim, Germany) were used throughout.

Digestion of PCR products. The use of dedicated restriction enzyme buffers was found to be unnecessary. Depending on the band intensity of the PCR products, they were digested undiluted or diluted 1:5 in sterile, double-distilled water. A 20-μl sample of PCR product containing 1 μg of DNA (or less) was mixed in a 1.5-ml reaction tube (Eppendorf) with either 1 μl of restriction enzyme *Taq*I (10 U) or 1 μl of *Ava*I and 1 μl of *Sfu*I (10 U each), and the mixture was incubated at 37°C for 90 min. The tubes were centrifuged briefly at least once during this time to prevent excessive condensation on the lid. Enzymes *Taq*I, *Ava*I, and *Sfu*I were obtained from Boehringer. Ten microliters of each digestion product was analyzed on 2% agarose as described above.

Adenovirus isolates for identification. Two hundred adenovirus isolates labelled only by sequential numbers (see Table 2) were sent from the adenovirus reference laboratory in Bilthoven, The Netherlands, at ambient temperature to Lund, Sweden, for blind testing. The virus isolates were crude cell culture fluid preparations. They included representatives of all human adenovirus subgenera and five simian adenoviruses. None of the 200 strains were cultured at the receiving laboratory prior to being tested by PCR.

Representation of *Taq*I profiles. The *Taq*I profiles shown below (see Fig. 5), corresponding to gel photographs, were generated by DNAman software (Lynnon Biosoft, Quebec, Canada) and are based largely on original sequence data from GenBank (Ad2 and Ad7 prototype strains) or VA RNA gene region sequences obtained by our group for selected adenovirus prototype strains (23) and wild isolates of Ad7 (20).

RESULTS

The test used in this study is based on differentiation between the VA RNA gene regions of the different human adenovirus subgenera by PCR. Adenoviruses of subgenera A, B:2, and F have one VA RNA gene (23, 24, 31) and yield PCR products ranging in size from 240 to 290 bp, whereas representative adenoviruses of subgenera B:1, C, D, and E, which have two VA RNA genes rather than one, yield products from 410 to 520 bp (Fig. 1). This affords the possibility of broad categorization of isolates into two main groups for further analysis.

(i) Differentiation between adenoviruses giving small (240- to 290-bp) PCR products. PCR subgenera A (240 bp), F (260 bp), and B:2 (290 bp) can be clearly distinguished from each other visually by electophoresis in 2% agarose. Thus, the sizes of PCR products alone allow differentiation between adenoviruses of these subgenera. If required, treatment of the PCR product with restriction enzyme *Sfu*I (which cleaves the subgenus A adenovirus product into two comigrating fragments of approximately 120 bp but fails to cut subgenus B:2 and F PCR products) combined with *Ava*I (which cleaves subgenus B:2 PCR products into two fragments of different sizes [approximately 130 and 160 bp] but fails to cut subgenus A and F PCR products) will confirm the subgenus identity of the isolate. Thus, an uncleaved product would indicate a subgenus F adenovirus.

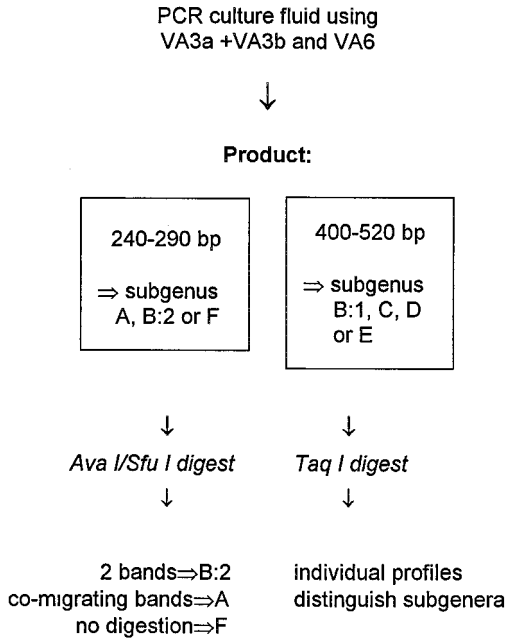

FIG. 2. Overall scheme for determination of the subgenus of an adenovirus isolate by PCR.

(ii) Differentiation between adenoviruses giving large (410- to 520-bp) PCR products. Differentiation of subgenus B:1, C, D, and E adenoviruses from each other involves a similar approach. Some information on subgenus can be gleaned from the size of the PCR product (since, for example, the PCR products of most, but not all, subgenus B:1 adenoviruses migrate the most slowly in agarose [> 500 bp]). As differentiation between subgenera B:1, C, D, and E on this basis is not reliable, we adopted a fingerprinting approach based on the presence of two similar genes. All VA RNA genes have promoter element B regions, and these usually include a TCGA motif (24, 31), which in Ad2 is thought to be important for interaction of the promoter with transcription factor TFIIIC (6). It is possible to fingerprint adenoviruses belonging to different subgenera by treating the amplified VA RNA region product with *Taq*I, which recognizes the TCGA motif. Adenoviruses with appreciably diverged genomes will have different distances between promoter element B motifs and hence between *Taq*I sites. In addition, those having VA RNA genes with one or more substitutions in this 4-base motif, which are not recognized by *Taq*I, should fingerprint as being genetically distinct.

Subgenus identification of prototype adenoviruses. A scheme summarizing the subgenus identification of adenovirus isolates by PCR is shown in Fig. 2. This approach was evaluated with the 49 known human adenovirus prototypes. All 47 established adenovirus prototypes and candidate Ad48 and Ad49 tested positive. Additionally, Ad4a and Ad11a, which are genetically distinct from their prototype counterparts (28, 29) and which may be agents of disease significantly distinct from their prototype equivalents, were positive in the test. The PCR was therefore tentatively regarded as a general test for all human adenoviruses isolated in culture.

Additionally, all subgenus A, B:2, and F prototypes, and also Ad11a of subgenus B:2, yielded short PCR products (240 to 290 bp). All such PCR products gave the expected results when digested with *Ava*I and *Sfu*I (Fig. 3). All larger PCR products (410 to 520 bp) came from subgenus B:1, C, D, or E adenoviruses. All subgenus C adenoviruses yielded the same pattern when digested with *Taq*I (Fig. 4), and the bands obtained (with *Taq*I fragment sizes of 201, 131, 120, and 53 bp predicted from the Ad2 genomic sequence) proved useful as molecular size markers against which other *Taq*I fragment sizes could be compared. The subgenus D adenoviruses gave two main *Taq*I profiles, depending on whether their VA RNA_{II} gene had a base substitution in the TCGA motif (23). Ad4 and Ad4a of subgenus E could be distinguished both by band size (Ad4a having a smaller product of approximately 410 bp compared with 460 bp for the Ad4 prototype) and by *Taq*I profile (four and six *Taq*I sites, respectively). The prototype Ad4 PCR product had the largest number of restriction sites for *Taq*I of all the adenoviruses tested. The prototype adenoviruses of subgenus B:1 gave three different profiles, which extended to six profiles after the testing of other clinical isolates (see below).

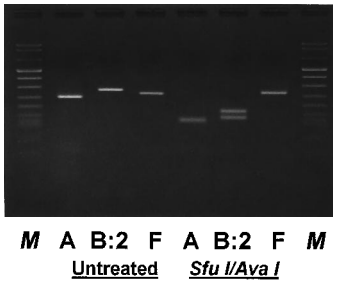

FIG. 3. Comparison of *Ava*I-*Sfu*I digest profiles for PCR products (240 to 290 bp) from adenoviruses of the indicated subgenera with undigested PCR products of the same subgenera. Lanes *M*, molecular size marker VIII (Boehringer) (see Fig. 1 legend).

Blind testing of adenovirus isolates. There can be considerable genetic variability among adenoviruses which have the same antigenic determinants (37, 38). It was thus considered important to carry out a wider survey of the scope of the adenovirus PCR, based largely on wild isolates, than would be possible by testing only the prototype strains. This assessment would give information on applicability of the PCR to adenoviruses in general and, since it was to be performed under code, also the accuracy of subgenus identification compared with conventional identification by neutralization. Two hundred adenovirus isolates in cell culture fluid, consisting mostly of wild strains belonging to all six human adenovirus subgenera but also including five simian adenoviruses, were tested blindly by PCR and identified by subgenus as described above. Nine of

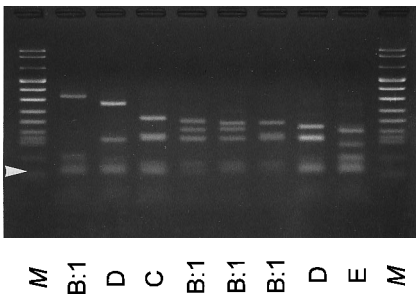

FIG. 4. Comparison of eight common *Taq*I digest profiles for PCR products from adenoviruses of the indicated subgenera. Not shown are two of the six known subgenus B:1 profiles and the Ad4a profile (illustrated in Fig. 5, lanes 3, 5, and 10, respectively). Excess primer was used here to illustrate that the positions of the lowest adenovirus DNA bands in all lanes (approximately 50 bp) almost coincide with, but are situated above, the position of primer dimer (arrowhead). Lanes *M*, molecular size marker VIII (Boehringer).

TABLE 2. Results of subgenus characterization of 200 adenovirus isolates by PCR

Subgenus from virus neutralization or primate source	No. of isolates				
	Not identified	Incorrectly identified	Partially identified correctly	Correctly identified	Total tested
A	0	0	0	13	13
B:1	0	0	0	22[a, b]	22
B:2	0	2[c]	1[d]	17	20
C	0	0	0	20	20
D	3[e, f]	0	8[g]	92[h]	103
E	0	0	2[i]	2	4
F	0	0	0	13	13
Chimp	0	0	0	1[j]	1
Monkey	0	1[k]	0	3	4
Total	3	3	11	183	200

[a] Six Ad7 isolates gave identical but previously unrecognized *Taq*I profiles. Any further isolates tested would be correctly identified.

[b] One subgenus B:1 isolate was untypeable by neutralization with antisera to Ad1 to Ad49.

[c] Two Ad14 (subgenus B:2) isolates (with Ad16 hemagglutinin) were characterized as subgenus B:1.

[d] The PCR product of one Ad34 isolate had no *Ava*I restriction site but the isolate was still identified as belonging to subgenus B:2 on the basis of the size of the PCR product.

[e] The quantities obtained of PCR products from one Ad8 isolate and one Ad13 isolate were too small for determination of subgenus.

[f] One Ad36 strain was PCR negative.

[g] Eight subgenus D isolates were characterized as belonging to either subgenus D or E.

[h] Eight subgenus D isolates were untypeable by neutralization with antisera to Ad1 to Ad47.

[i] Two isolates of Ad4 were characterized as belonging to either subgenus D or E.

[j] All chimpanzee adenoviruses fall into human adenovirus subgenera, in this case B:1.

[k] One monkey adenovirus strain was identified as belonging to subgenus F.

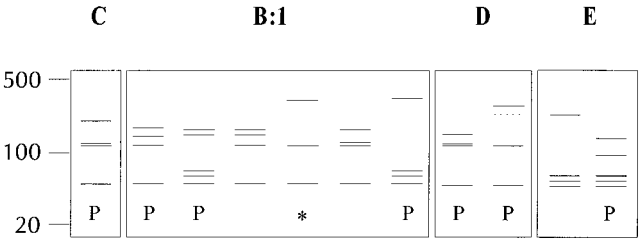

FIG. 5. Diagrammatic representation of all subgenus B:1, C, D, and E adenovirus PCR products spanning the VA RNA gene region, digested with *Taq*I endonuclease. Numbers on the left indicate fragment sizes (in base pairs). P, profile associated with prototype strains; *, rare profile associated with a representative of the Ad7f genome type. The dotted line represents the position of the largest *Taq*I fragment seen for a small number of subgenus D strains (including prototype Ad10). Note that the Ad4a profile (subgenus E, left lane) has no band in the 100- to 200-bp range, which allows differentiation from prototype Ad10. The subgenus C profile, as represented by Ad2, serves as an excellent standard for comparison in all determinations.

the 195 human adenovirus isolates had not been typeable as Ad1 to Ad47 by the laboratory at Bilthoven using neutralization tests; however, eight of these had been recognized as belonging to subgenus D and one as belonging to subgenus B:1 by various other criteria. The results of the PCR are shown in Table 2. These results refer to culture fluids tested undiluted, or, in the event of a weak PCR product initially, culture fluids retested at a dilution of 1/10.

Six of the 200 isolates (3%) yielded negative or erroneous results. First, three isolates of subgroup D (strains of Ad8, Ad13, and Ad36) either consistently failed to yield PCR products (the Ad36 strain) or yielded products which were too weak to be useful in *Taq*I digests. Of the three results which were apparently wrong, two involved a hemagglutination variant. These adenovirus isolates carried the neutralization antigen(s) of Ad14 and therefore belong to type 14 (subgenus B:2), but they also carried the hemagglutination antigen(s) of Ad16 (subgenus B:1). These two adenovirus isolates were found to carry subgenus B:1 VA RNA genes. The inaccuracy in this case may have been due to chimeric Ad16 genomes carrying the hexon gene of Ad14. Second, one monkey adenovirus strain gave a PCR product matching the size of those from subgenus F adenoviruses. As this product was not digested by *Sfu*I and *Ava*I in combination, the strain was mistakenly identified as belonging to subgenus F. A high level of DNA sequence identity between the VA RNA regions of subgenus F adenoviruses and two strains of monkey adenovirus (of types SAV16 and SAV19) has been noted previously (23, 24).

Eleven further isolates (5.5%) were identified partially correctly. Similar to the subgenus F adenoviruses, one strain of Ad34 had no *Ava*I site in its PCR product of 290 bp and was only tentatively reported as belonging to subgenus B:2. Another 10 partially correct identifications involved difficulties in differentiating certain subgenus D strains from Ad4a of subgenus E, and for this reason they were reported as subgenus D or E. After the blind trial, the *Taq*I profiles for subgenera D and E were carefully compared (Fig. 5, lanes 9 and 10). Subgenus D and Ad4a can be distinguished reliably on the basis of the presence of a 120-bp digestion product for subgenus D only (Fig. 5).

One hundred eighty-three isolates (91.5%) were correctly identified. Although six of them had *Taq*I profiles which were recognizably identical and were reported as such, they had not been encountered in the work with prototypes. Hence, they are arguably scored in Table 2 as being correctly identified rather than partially identified since the *Taq*I-digested PCR products of these subgenus B:1 strains were wholly distinct from all other strains of this subgenus (Fig. 5, lane 6). All six isolates had been typed as Ad7 by neutralization. A fifth *Taq*I profile for subgenus B:1 adenoviruses was also encountered and correctly identified during the blind testing (Fig. 5, lane 4).

Investigation of subgenus B:1 genome types. The VA RNA gene region polymorphism shown by wild strains of subgenus B:1 and, in particular, those identified as Ad7 by neutralization prompted us to investigate the extent of VA RNA gene variation in known subgenus B:1 genome variants. *Taq*I profiles of amplified VA RNA gene regions were obtained for Ad7b, Ad7c, Ad7d, Ad7e, Ad7f, Ad7g, Ad7h, and Ad7i. One new Ad7 VA RNA gene region profile (corresponding to the uncommon Ad7f) was seen in addition to the five subgenus B:1 *Taq*I profiles already obtained. Representatives of Ad7b, Ad7c, Ad7d, Ad7h, and Ad7i had the same *Taq*I profile (Fig. 5, lane 6). Prototype Ad7 (Ad7p) and Ad7g shared a profile (Fig. 5, lane 3), whereas Ad7e and Ad7f each had a unique *Taq*I profile (Fig. 5, lanes 4 and 5). Subsequently, strains of Ad3p2, Ad3a, Ad3c, Ad3d, Ad16w, Ad16x, Ad21a, and Ad21b strains were also tested, but no new subgenus B:1 profiles were obtained in addition to the six already seen. However, it was clear from this work that exchange of genetic material between members of subgenus B:1 has most likely been common. The serotype identity of a B:1 isolate by neutralization (as Ad3, Ad7, Ad16, or Ad21) has little or no correlation with VA RNA gene region identity as determined by PCR amplification and *Taq*I digestion. Thus, identification of one of the six subgenus B:1 profiles can be used only for subgenus and cluster identi-

fication and not for identification of serotype or of specific genome variants.

DISCUSSION

Identification of adenovirus isolates on the basis of subgenus would be inadequate for epidemiological surveillance, which generally involves typing by neutralization tests and perhaps finer differentiation between genomic variants using DNA analysis with restriction enzymes. However, in the clinical setting, identification of adenoviruses by subgenus alone is sufficient. Generally, the adenovirus types within a subgenus are similar in tropism, pathogenic potential, tendency to latent infection, and occurrence or reactivation in immunosuppressed individuals (Table 1). On the basis of DNA analysis, adenoviruses of subgenus B fall into two clusters, B:1 and B:2, the members of which generally show different tropisms. Fortunately, the two clusters can be distinguished by the present method of subgenus identification.

There is some evidence that different strains of certain adenovirus serotypes may have different tropisms. For example, there exists a variant of Ad11 (Ad11a) which has been isolated mainly from the respiratory tract, whereas strains most related to the Ad11 prototype (Ad11p) are usually isolated from urine (29). Ad4 also shows considerable genetic variation (28), and these genomic differences may correlate with variation in tropisms between strains (38). Thus, some Ad4 strains may be associated mainly with respiratory symptoms, while others cause mainly eye infections which may manifest as acute hemorrhagic conjunctivitis. Such differences in tropism, if true, must somehow be due to genomic variation (perhaps involving several parts of the genome) among adenoviruses carrying the same antigenic determinants. In our opinion, the existence of such adenovirus strains does not detract from the general rule that identification by subgenus is sufficient in clinical practice. Subgenus identification is at least as sufficient as typing by neutralization, which cannot distinguish between Ad11 and Ad11a or between Ad4 and Ad4a.

On the other hand, adenovirus strains with intermediate phenotypes exist in nature. By comparing the results of neutralization and hemagglutination inhibition tests, strains with the characteristics of two different serotypes can be detected, for example Ad11/H14 (16) and Ad14/H16 (34) of subgenus B and Ad15/H9 (1) and Ad32/H27 (9) of subgenus D. These variants usually arise through recombination between adenoviruses with appreciable genome homology (7), such that the parental strains belong to the same subgenus. Both of these phenomena indicate that serotype classification, while acting as a more specific label for a virus isolate, may be no more meaningful for clinical diagnosis than subgenus classification.

The PCR method used here has advantages beyond subgenus determination, which should also make it useful for epidemiological work. With the exception of subgenus D adenoviruses, the subgenus identification of an adenovirus isolate can facilitate serotype identification by neutralization. Thus, once the subgenus of an adenovirus strain is defined by PCR, serotyping or serotype confirmation would involve the use of only one to four neutralizing antisera for adenoviruses in subgenera A, B:1, B:2, C, E, and F.

An Ad14/H16 strain as described above was encountered in this study during the testing of clinical isolates. While this strain poses no difficulty for establishing its subgenus identity, different parts of this adenovirus genome are clearly derived from parent adenoviruses representing different subgenus B clusters. Because of the existence of this recombinant and possibly other rare subgenus B:1-B:2 intertypic recombinants, cluster identification of subgenus B isolates as B:1 or B:2, once established by PCR, would best be confirmed by neutralization tests using the four relevant antisera (either anti-Ad3, Ad7, Ad16, and Ad21 or anti-Ad11, Ad14, Ad34, and Ad35, depending on the presumptive PCR identification).

The results of blind identification of adenovirus isolates have highlighted an unexpected heterogeneity among the VA RNA gene regions of wild isolates of subgenus B:1. We have identified six different *Taq*I profiles in amplified DNA from either prototype strains or wild isolates of subgenus B:1, and well-characterized genome variants of Ad3, Ad7, Ad16, and Ad21 were also seen to show the same range of profiles. This illustrates the necessity of taking wild strains of adenovirus into account when assessing any new test based on genomic sequences. The reason for genomic sequence heterogeneity around map unit 30 among members of subgenus B:1 in particular is unclear, especially since so little variation was seen among the 31 known members of subgenus D.

We cannot exclude the possibility that there exist in nature other adenovirus strains whose VA RNA gene regions do not match the restriction enzyme profiles seen in this study. Our survey of wild adenovirus strains was not exhaustive, and we especially recommend conventional typing of any strains giving PCR products of 410 to 520 bp which show abnormal *Taq*I profiles different from those depicted in Fig. 5. However, it should be noted that PCR products at high concentrations can be incompletely digested, especially if the enzyme has lost activity upon storage. As poor DNA cleavage is a much more likely explanation for an abnormal digestion profile, dilution of the concentrated PCR product in the appropriate restriction enzyme buffer before *Taq*I analysis should always be attempted in cases of uncertainty.

Our assessment of this PCR has largely involved its use as a means of characterizing adenovirus isolates. However, some adenoviruses, such as those of subgenus F, are notoriously difficult to passage in culture, and the direct testing of clinical specimens may be an advantage in such cases. The method has been extended successfully recently to include rapid testing of transport medium from eye swabs (1 μl) without any form of specimen extraction, which will be reported separately. This provides a means of differentiating subgenus B:1 and subgenus D adenovirus infections of the eye, as amplified DNAs from the two subgenera are of sufficiently different sizes to be differentiated visually in gels. This differentiation between adenovirus infections can be important in ophthalmic practice. Ocular infections with B:1 adenoviruses are relatively harmless, whereas nosocomial spread of subgenus D adenoviruses, which can cause severe keratitis, may necessitate the temporary closing of an ophthalmic unit. Thus, in certain situations, it might be possible to dispense with both virus culture before the PCR and restriction enzyme digestion after the PCR and achieve a useful and clinically meaningful result within 24 h.

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