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Multiplexed Luminex xMAP Assay for Detection and Identification of Five Adenovirus Serotypes Associated with Epidemics of Respiratory Disease in Adults▽†

Cicely Washington,[1]* David Metzgar,[2] Manzour Hernando Hazbón,[1] Leonard Binn,[1] Arthur Lyons,[1] Carl Coward,[1] and Robert Kuschner[1]

Division of Viral Diseases, Walter Reed Army Institute of Research, Silver Spring, Maryland 20910,[1] and Department of Respiratory Diseases Research, Naval Health Research Center, San Diego, California 92152[2]

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Several serotypes of human adenovirus (HAdV) cause acute respiratory disease (ARD) among healthy adults, sometimes generating broad outbreaks with high attack rates and occasional fatalities. Timely serotype identification provides valuable epidemiological information and significantly contributes to prevention (vaccination) strategies. The prevalence of specific serotypes causing ARD varies geographically. HAdV-3, HAdV-4, HAdV-7, HAdV-14, and HAdV-21 are the serotypes most commonly found in adult populations in the Western Hemisphere. Unfortunately, conventional serotype identification is a tedious process which can take a week or longer. For this reason, new molecular methods for serotype identification are needed. Commercially available rapid antigen and PCR assays for the detection of HAdV are universal but do not distinguish between the different serotypes. We describe the development of a sensitive and specific multiplex assay capable of identifying serotypes 3, 4, 7, 14, and 21. Two sets of primers were used for nonspecific (universal) PCR amplification, and serotype-specific probes coupled to Luminex tags were used for target-specific extension (TSE). PCR and TSE primers were designed using known hexon gene sequences of HAdV. The TSE products of HAdV-3, HAdV-4, HAdV-7, HAdV-14, and HAdV-21 were correctly identified using the Luminex xMAP fluid microsphere-based array system. No cross-reactivity with other respiratory pathogens or other HAdV serotypes was observed. This multiplexed assay can be expanded to include more serotypes and will allow broad and rapid detection and identification of adenoviral serotypes in a high-throughput environment.

Human adenoviruses (HAdVs) cause a wide range of diseases in humans, including upper and lower respiratory illness, urinary tract infections, conjunctivitis, and gastroenteritis. There are 51 different serotypes based on type-specific serum neutralization, and these are classified into six species (A, B, C, D, E, and F) on the basis of hemagglutination, oncogenic, and phylogenetic properties (13, 24, 28, 31).

The most common serotypes known to cause respiratory illness in the adult population are 3, 4, 7, 11, 14, and 21 (21, 24, 25, 37). All of these can cause locally severe outbreaks with high attack rates. These types of outbreaks are rarely reported in civilian populations but are essentially continuous at military training camps, particularly with serotype 4 (HAdV-4) and, to a lesser extent, HAdV-7 (10). Some recent studies have suggested that specific serotypes cause more severe disease, especially in immunocompromised patients (8, 13, 14, 24, 28, 32). The military previously established universal vaccination of new recruits for HAdV-4 and -7, which reduced adenovirus-induced acute respiratory disease (ARD) by 95 to 99% (20). Production of these vaccines ceased in 1996, but similar replacement vaccines are currently in the final stages of licensure. In 2006 and thereafter, HAdV-14 and HAdV-21 produced extensive outbreaks of ARD in military recruits (20).

It is vital to be able to identify the serotype and changes in the serotype over time in order to evaluate viral virulence, vaccine efficacy, and the potential impact of antiviral use. Identification of serotypes was traditionally done by virus isolation in cell culture, followed by neutralization tests, antibody studies, and/or antigen detection by immunofluorescence (3, 16, 19, 23). These techniques are time-consuming and labor-intensive (11, 34). Another technique that has been used is whole-genome restriction endonuclease analysis, which relies on large-scale viral culture to generate the full genomic substrate (2, 4, 5).

More recently, PCR-based detection and discrimination methods have been developed (1, 21, 35, 36). These techniques are faster and can also detect coinfections when used in a multiplex assay (20, 21, 33, 35, 36), thus reducing cost, labor, and sample volume needed for analysis. Current PCR assays identify the six subgenera (A to F) or up to three serotypes per reaction mixture (1, 21, 33, 35, 36). Real-time PCR also exists for generic detection (but not discrimination) of all 51 serotypes (6, 7, 12, 21, 33), and sequence analysis of the genomic region coding for the seven hypervariable loops of the hexon (the primary antigenic determinant) can identify and discriminate all 51 serotypes with a single assay (27), albeit a relatively time-consuming and complex one.

Luminex has designed an xMAP system that in theory can detect up to 100 pathogens in a single sample by coupling

* Corresponding author. Mailing address: 503 Robert Grant Ave., Silver Spring, MD 20910. Phone: (301) 319-9824. Fax: (301) 319-9661. E-mail: Cicely.Washington@us.army.mil.

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TABLE 1. Coordinates and sequences of amplification and TSE primers used in the study

PCR primer[a]	Sequence	TSE primer	Sequence
HVR7′, forward[b,c]	1003-CTGATGTACTACAACAGCACT GGCAACATGGG-1033	HAdV-3[d]	2616-GTTAAAACCGATGACACTAATGG-2638
HVR7, reverse[b,c]	1575-CGGTGGTGGTTAAATGGATTC ACATTGTCC-1604	HAdV-4[e]	19382-GGTGTGGGATTGACAGACACTTAC-19405
HAdV-7, forward	383-CGCCCAATACATCTCAGTGG-402	HAdV-14[f]	19544-CCAAGCTTGGAAAGATGTAA ATC-19566
		HAdV-21[c]	1299-GGGTGCAGATTGGAAAGAGC-1318
HAdV-7, reverse	595-ACTCCAACTTGAGGCTCTGG-614	HAdV-7[g]	399-GTGGATAGTTACAACGGGAGAAG-421

[a] Amplicon sizes for the PCR primers were 601 bp (HVR7 forward and reverse primers) and 253 bp (HAdV-7 forward and reverse primers).
[b] Primer from Sarantis et al. (27).
[c] Nucleotide numbering is based on the hexon gene of HAdV accession number AY008279.
[d] Nucleotide numbering is based on the hexon gene of HAdV accession number AY599834.
[e] Nucleotide numbering is based on the hexon gene of HAdV accession number AY599837.
[f] Nucleotide numbering is based on the hexon gene of HAdV accession number AY803294.
[g] Nucleotide numbering is based on the hexon gene of HAdV accession number AY594255.

bioassays with digital signal processing in real time. The platform is a suspension array where specific capture moieties are covalently coupled to the surfaces of internally dyed microspheres (22). The diversity of these microspheres increases the number of targets that can be identified in a single sample approximately 20-fold over traditional real-time PCR.

In this study we designed and tested a Luminex-based assay capable of detecting and identifying HAdV-3, HAdV-4, HAdV-7, HAdV-14, and HAdV-21 in a single reaction. The assay was tested, and the results indicate its potential as a diagnostic tool.

MATERIALS AND METHODS

Sample collection and initial identification. Clinical specimens were collected by the Naval Health Research Center (NHRC) under an Institutional Review Board-approved protocol (NHRC.1999.0002) with support from the Armed Forces Health Surveillance Center/Division of Global Emerging Infections Surveillance and Response System under work unit 60805. Inclusion criteria for subjects who consented and enrolled through the NHRC febrile respiratory illness surveillance system included military recruits reporting for medical care with respiratory symptoms and a fever of ≥38°C, provider-diagnosed pneumonia, or both. Specimens were oropharyngeal swabs suspended in viral transport medium (VTM; Copan Diagnostics Inc., Murrieta, CA) and subsequently frozen at −80°C and transported on dry ice for testing. The presence of HAdV serotypes 3, 4, 7, 14, and 21 was initially identified in 78 of these specimens at NHRC by using a modified colorimetric microneutralization assay (19), PCR, or both methods, as described previously (20).

Viral strains and isolates. HAdV isolates used in this study were part of the strain bank from the Division of Viral Disease (DVD) at Walter Reed Army Institute of Research (WRAIR) and were grown using A549 cells as previously described (15, 26). The following HAdV strains were used: HAdV-1 (Adenoid 71), HAdV-2 (Adenoid 6), HAdV-3 (GB), HAdV-4 (RI-67), HAdV-5 (Adenoid 75), HAdV-6 (Tonsil 99), HAdV-7a (S-1058), HAdV-7 (Gomen), HAdV-9 (Hicks), HAdV-11 (Slobitski), HAdV-14 (DeWit), HAdV-16 (CH76), HAdV-17, HAdV-21 (strain 128), HAdV-31 (1315/63), HAdV-34 (Compton), HAdV-35 (Holden), and HAdV-40 (Dugan). Cultures from strains GB, RI-67, S-1058, Gomen, DeWit, and 128 HAdV were titrated in tube cultures for 21 days, and titers were expressed as the 50% tissue culture infective dose ($TCID_{50}$).

Other common respiratory pathogens were obtained using the NATrol respiratory validation panel (Zeptometrix, Buffalo, NY). This panel includes coronavirus OC43, severe acute respiratory syndrome (SARS) corona virus, influenza A virus H1N1 and H3N2, influenza B virus, parainfluenza virus 2 and 3, adenovirus 7a, metapneumovirus, respiratory syncytial virus (RSV) A and B, enterovirus, and rhinovirus strains.

DNA extraction. Oropharyngeal swabs and cultured isolates were processed under biosafety level 2 conditions at the DVD, WRAIR. DNA was isolated using the MinElute virus spin kit (Qiagen, Valencia, CA) according to the manufacturer's recommendations. The sample and elution volumes were 200 μl. Sample extracts were stored at −70°C.

PCR primer and TSE primer design. For PCR and target-specific extension (TSE) primer design, eight hexon gene sequences from the serotypes of interest (GenBank accession numbers AY599834, AY599836, AY599837, AY594255, AF065066, AY495969, AY80329, and AY008279) were ClustalW aligned by using DNAStar Lasergene 8.0 software. A primer pair previously described was used to amplify a 605-bp-long region of the hexon gene (27). A set of serotype-specific TSE primers was designed to bind serotypes 3, 4, 14, and 21. In the case of serotype 7, a separate PCR primer pair was designed with its corresponding TSE primer (Table 1).

The description of the primers, including the position, sequence, and amplicon size are described in Table 1. The same alignment used to design the PCR primers was used to design the TSE primers. TSE primers were chosen to have a melting temperature between 50°C and 56°C.

PCR amplification. The multiplex PCR was performed using the Multiplex PCR kit (Qiagen, Valencia, CA). The reaction mixture contained 12.5 μl of 2× master mix buffer, one of four primers (2 pmol of each; Sigma Genosys, The Woodlands, TX), sample (5 μl), and water (6.7 μl) to produce a final volume of 25 μl. The resulting mixture was then cycled in a GeneAmp PCR system 9700 thermal cycler (Applied Biosystems, Foster City, CA) with an initial denaturation cycle at 95°C for 15 min followed by 35 cycles at 94°C for 30 s, 52°C for 1.5 min, and 72°C for 1 min, and a final incubation at 72°C for 10 min.

The resulting PCR product was then treated with shrimp alkaline phosphatase (3.125 μl; USB, Cleveland, OH) and exonuclease (2.5 μl; USB) at 37°C for 30 min followed by 99°C for 30 s to remove the remaining deoxynucleoside triphosphates and primers.

Target-specific primer elongation. The reaction mixture for target-specific primer elongation contained 10× Qiagen PCR buffer (2 μl), 50 mM $MgCl_2$ (0.5 μl), *Tsp* polymerase (0.15 μl of a 5-U/μl solution; Invitrogen, Carlsbad, CA), dATP (0.1 μl of 1 mM; Invitrogen), dGTP (0.1 μl of 1 mM; Invitrogen), dTTP (0.1 μl of 1 mM; Invitrogen), biotin-dCTP (0.25 μl of 4 mM; Invitrogen), one of five TSE primers (0.125 μl of 1 μM; Sigma), treated PCR product (5 μl), and water (11.2 μl) to produce a final volume of 20 μl. The resulting mixture was then thermocycled for an initial cycle at 95°C for 2 min, followed by 40 cycles at 94°C for 30 s, 55°C for 1 min, and 74°C for 2 min.

Hybridization and Luminex analysis. Biotinylated TSE products were hybridized to a fluid microbead array in wells of a 96-well plate and detected using a streptavidin-phycoerythrin conjugate. The microsphere mix consists of five types of microspheres, each containing a different fluorescent dye mix and each coupled to a unique anti-tag oligonucleotide sequence complementary to the oligonucleotide tag sequence incorporated into the five TSE primers. TSE product (12.5 μl) and H_2O (12.5 μl) were mixed with microsphere mix (25 μl; 2,500 microspheres per set) and incubated (at 96°C for 2 min and then 37°C for 30 min). After hybridization the plate was centrifuged (at 2,250 × *g* for 3 min) and the supernatant removed. Streptavidin-phycoerythrin (2 μg/ml) in 1× Tm (0.1 M Tris-HCl [pH 8.0], 0.2 M NaCl, 0.08% Triton X-100) was added to each well. The plate was incubated (at 37°C for 15 min) in the dark. Hybridized microspheres were then analyzed on the Luminex 200 at 37°C. TSE products bound to the microspheres were detected with a streptavidin-phycoerythrin conjugate, and signals produced for each bead were analyzed by using the Luminex 200 and expressed as the mean fluorescence intensity (MFI) (18). Any signal that was greater than three times the highest background MFI signal for a given bead set was considered a positive call.

TABLE 2. Assay specificity and sensitivity[a]

Viral pathogen(s)	TSE signal/lowest detected $TCID_{50}$ for HAdV serotype:					Luminex RVP assay call[b]
	3	4	7	14	21	
HAdV-3	$+/5 \times 10^3$	−	−	−	−	Adenovirus
HAdV-4	−	$+/9.5 \times 10^3$	−	−	−	Adenovirus
HAdV-7	−	−	$+/1.1 \times 10^4$	−	−	Adenovirus
HAdV-7a	−	−	+/ND	−	−	Adenovirus
HAdV-14	−	−	−	$+/5 \times 10^3$	−	Adenovirus
HAdV-21	−	−	−	−	$+/4.6 \times 10^5$	Adenovirus
HAdVs 3 and 7	+	−	+	−	−	NA
HAdVs 4, 14, and 21	−	+	−	+	+	NA
HAdVs 3, 4, 7, 14, and 21	+	+	+	+	+	NA
HAdVs 1, 2, 5, 6, 9, 11, 16, 17, 31, 34, 35, and 40	−	−	−	−	−	ND
Influenza A virus H1N1	−	−	−	−	−	Influenza A virus H1
Influenza A virus H3N2	−	−	−	−	−	Influenza A virus H3
Influenza B virus	−	−	−	−	−	Influenza B virus
Coronavirus OC43	−	−	−	−	−	Coronavirus OC43
Coronavirus SARS	−	−	−	−	−	Coronavirus SARS
Parainfluenza virus 2	−	−	−	−	−	Parainfluenza virus 2
Parainfluenza virus 3	−	−	−	−	−	Parainfluenza virus 3
RSV A	−	−	−	−	−	RSV A
RSV B	−	−	−	−	−	RSV B
Metapneumovirus	−	−	−	−	−	Metapneumovirus
Enterovirus	−	−	−	−	−	Entero-/rhinovirus
Rhinovirus	−	−	−	−	−	Entero-/rhinovirus
Blank	−	−	−	−	−	−

[a] Assay specificity was assessed by testing with the pathogens indicated. Assay sensitivity was assessed by determining the lowest dilution detected from serial dilutions for each reference HAdV strain tested (see the text for details). The corresponding $TCID_{50}$ of the lowest dilution detected is indicated. The coinfections were not tested by a commercial method because they do not distinguish serotypes.

[b] NA, not available. ND, samples were not tested with the Luminex RVP kit but rather with the Argene adenovirus r-gene kit.

RESULTS

In this study we describe the design and evaluation of a multiplexed assay capable of detecting and identifying HAdV-3, -4, -7, -14, and -21. The original assay design consisted of an amplification of a single region from the hexon gene. This region was selected because the sequences where the PCR primers hybridize are conserved among different serotypes of HAdVs but the areas in between them vary between serotypes, allowing a selective binding for TSE primers designed to specifically bind each serotype. With the exception of serotype 7, this approach was successful. Several TSE primers designed to identify serotype 7 showed cross-reactivity with serotype 3 (data not shown). In order to circumvent this, the final assay design uses a combination of two PCR primer pairs, one that amplifies the target sequences for serotypes 3, 4, 14, and 21 and the second for serotype 7 (Table 1). With this assay design, we observed TSE signals only in the presence of the corresponding serotype, without cross-reactivity between TSEs. The assay was further tested with a combination of two HAdV serotypes in a single reaction. The assay was able to identify the two serotypes present in the reaction mixture (Table 2).

Analytical specificity and sensitivity. The specificity of the multiplexed assay was examined by testing in triplicate 30 different pathogens (including the common respiratory pathogens found on the NATrol Respiratory Validation Panel [Zeptometrix, Buffalo, NY] and 18 HAdV serotypes, coronavirus OC43, coronavirus SARS, influenza A H1N1 and H3N2, influenza B, parainfluenza 2 and 3, adenovirus 7a, metapneumovirus, respiratory syncytial virus A and B, enterovirus, and rhinovirus). In addition, isolates of the following adenovirus serotypes were tested: HAdV-1 (Adenoid 71), HAdV-2 (Adenoid 6), HAdV-3 (GB), HAdV-4 (RI-67), HAdV-5 (Adenoid 75), HAdV-6 (Tonsil 99), HAdV-7a (S-1058), HAdV-7 (Gomen), HAdV-9 (Hicks), HAdV-11 (Slobitski), HAdV-14 (De-Wit), HAdV-16 (CH76), HAdV-17, HAdV-21 (strain 128), HAdV-31 (1315/63), HAdV-34 (Compton), HAdV-35 (Holden), and HAdV-40 (Dugan), as shown in Table 2. All our reference adenoviruses were prepared in A549 cells and were harvested when the cell cultures developed ≥75% cytopathic effect. After harvesting, virus preparations were titrated in tube cultures for 21 days, and titers are expressed as the $TCID_{50}$. All samples were extracted following the protocol above. In order to confirm the presence of HAdV in the extracted cultured isolates, the Adenovirus r-gene kit (Argene, North Massapequa, NY), a universal adenovirus PCR-based kit, was used according to the manufacturer's protocol. The assay was able to detect all adenovirus serotypes tested (Table 2). To further test the performance of the assay, the presence of multiple targets was tested in combination (Table 2). The ability to detect mixed HAdV infections is valuable, because they do occur naturally and are difficult to detect by many methods (30).

Having determined the assay specificity, the limit of detection for each of the detected serotypes was determined. This was achieved by testing in duplicate five serial dilutions (10-fold each) of cultured isolates of serotypes 3, 4, 7, 14, and 21. The virus isolates were prepared in A549 cells. They were harvested when the cell cultures developed 75% or more cytopathic effect (CPE). After harvesting the virus preparations,

TABLE 3. Comparison of PCR/microneutralization and Luminex HAdV assay results obtained at NHRC[a]

HAdV serotype	No. of samples with indicated results in the two assays				Sensitivity (%)	Specificity (%)
	W+ N+	W− N+	W+ N−	W− N−		
3	9	1	0	94	90	100
4	19	0	2[b,c]	83	100	98
7	10[b]	0	0	94	100	100
14	19[c]	0	0	85	100	100
21	17	3	0	84	85	100

[a] Comparison of the results obtained by PCR/microneutralization and with the Luminex HAdV assay. W, result (+ or −) by the multiplexed Luminex HAdV assay; N, result (+ or −) by PCR/microneutralization. Sensitivity and specificity were based on true positives and true negative, defined as specimens giving positive or negative results according to the PCR/microneutralization assay. The sensitivity and specificity were calculated according to the methods reported in Cumitech 31 (9).
[b] One HAdV-7 sample was positive for both HAdV-4 and HAdV-7.
[c] One HAdV-14 sample was positive for both HAdV-14 and HAdV-4.

they were titrated in tube cultures for 21 days, and titers are expressed as the $TCID_{50}$ (16, 26). The lowest dilutions in which the TSEs were able to detect the presence of the viruses are shown in Table 2.

Evaluation of clinical specimens. In order to evaluate the assay's performance using clinical specimens, we tested 104 respiratory samples previously determined to be positive for HAdVs of the target serotypes. These samples were tested blindly in triplicate. The reproducibility (agreement levels) for TSE primers for HAdV-3, HAdV-4, HAdV-7, HAdV-14, and HAdV-21 was 99%, 93%, 94%, 94%, and 96%, respectively. The results obtained are shown in Table 3. A total of five discrepancies between the Luminex results and the original results were observed and were equal to 5% of the total tested. One positive HAdV-3 sample and three positive HAdV-21 samples were negative by our assay, while a specimen previously identified as HAdV negative tested positive for HAdV-4 with our assay. These five discrepant specimens were tested with the Luminex RVP kit (17), and HAdV was detected in all but one of these specimens (the exception being a specimen that was positive for HAdV-21 by the PCR/microneutralization assay). Two samples identified as coinfections by the custom Luminex assay were initially identified as single infections by the PCR/microneutralization assays (Table 3). Both samples were HAdV-4 positive. One was also positive for HAdV-7 and the other for HAdV-14 (see Table S1 in the supplemental material).

DISCUSSION

Adenovirus is a common cause of ARD in military recruit populations. There are five major serotypes that affect U.S. military training camps. By using Luminex technology, we have developed a sensitive and specific multiplexed PCR assay that can detect and identify the five relevant serotypes, HAdV-3, HAdV-4, HAdV-7, HAdV-14, and HAdV-21. The assay targets the hexon gene, because the conserved nature of specific structural regions among all serotypes allows design of universal primers, while the diversity of intervening hypervariable regions can be used to distinguish serotypes with differential probes. One hundred four clinical throat swab samples previously identified as positive or negative for serotypes HAdV-3, HAdV-4, HAdV-7, HAdV-11, HAdV-14, and HAdV-21 were tested. The assay exhibited high sensitivity and specificity in both analytical and clinical specimens. When challenged with various other common respiratory viruses and adenovirus serotypes, no cross-reactivity was detected. With the reintroduction of the vaccine program in the military recruit population, there are fears that different serotypes may emerge as the dominant agents of ARD outbreaks. The ability to quickly identify shifts in serotype dominance will enable a more informed assessment of the vaccine's efficacy. Past studies have suggested that there is an increase in levels of neutralizing antibodies in serum against HAdV-3 and HAdV-14 after HAdV-7 immunization (20, 29). Analysis of the vaccine's efficacy against both homotypic and heterotypic HAdVs will certainly require an efficient and robust method for measuring the impact of the vaccine on the rates of disease associated with specific serotypes.

With multiplexed molecular assays there are concerns of decreased sensitivity and specificity related to the multiplicity of primers and probes and the possible combinations thereof. Primers and probes for each target may interfere with one another by forming dimers and/or by nonspecific partial binding to target sequences. This can be minimized by optimizing primer and probe design, most importantly by appropriate use of sequence conservation and variability among the targets. This assay was designed to minimize the number of oligonucleotides in the reaction through the use of broadly targeted primers designed to amplify multiple targets, paired with highly specific probes that identify individual serotypes. Based on the 104 clinical samples tested, the sensitivity for the different serotypes tested was 90%, 100%, 100%, 100%, and 85% for HAdV-3, HAdV-4, HAdV-7, HAdV-14, and HAdV-21, respectively (Table 3). It is possible that a new design for the HAdV-21 TSE primer could increase the level of sensitivity further.

The results showed five discrepant results with the original calls obtained at NHRC. Three of the discrepant results were cases in which HAdV-21 was not detected by the Luminex assay. We observed that the TSE primer for serotype 21 showed the lowest sensitivity (4.6×10^5 $TCID_{50}$). This was the likely source of these false negatives. The Luminex RVP kit was used to test those samples, and HAdV was detected in two of the three (see Table S1 in the supplemental material), suggesting that one could be a real negative, while the others were confirmed as false negatives. The apparent false-positive HAdV-4 result, detected as HAdV by both the novel Luminex test described here and the Luminex RVP kit (see Table S1) is most likely real. All of the negative samples were obtained

from U.S. military recruit populations, in which HAdV-4 is essentially endemic. This discrepancy is likely a false negative on the part of the original PCR/microneutralization tests.

The new assay was capable of detecting coinfections of multiple adenovirus serotypes in clinical specimens as well as in artificial mixtures of cultured isolates (Tables 2 and 3). In our study, two throat swab specimens were found to be coinfected, one with HAdV-4 and HAdV-14 and the other with HAdV-4 and HAdV-7. Previously, these were determined to be HAdV-14 and HAdV-7 by PCR testing. Resolution of these discrepant results would require confirmatory testing with additional molecular tests. Previous work by Vora et al. (30) indicated a high rate of coinfections of HAdV-4 and HAdV-7, and the observation of them in the sample set tested here is not surprising since they are the two most common ARD-associated serotypes in recruit populations (30). Coinfections are harder to detect, because one serotype, usually the one with a higher titer, often dominates detection assays, especially in the case of single-pass comparative immunological methods. Coinfections are of interest because they provide the opportunity for adenovirus strains to recombine and possibly form new variants. Our assay has the ability to detect coinfections in a single test.

The results in this study indicate that the assay described has potential as a diagnostic tool. It is simple, the cost is relatively low, and it provides for the identification of up to five HAdV serotypes in a single reaction. The assay can be expanded to include more serotypes as needed. For example, pediatric and immunocompromised patients tend to experience infections of group C HAdV serotypes. Current efforts are under way to include group C serotypes HAdV-1, HAdV-2, HAdV-5, and HAdV-6.

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