Performance Characteristics of FilmArray Respiratory Panel v1.7 for Detection of Adenovirus in a Large Cohort of Pediatric Nasopharyngeal Samples: One Test May Not Fit All

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The FilmArray Respiratory Panel (RP) v1.7 assay has improved sensitivity for detection of human adenovirus (HAdV), compared to an earlier version (RP v1.6). RP v1.7 was designed for detection of species B, C, and E but may show variable detection of species A, D, and F. We sought to evaluate the clinical and analytical performance of RP v1.7 for detection of HAdV in a large pediatric cohort. Respiratory specimens obtained from a tertiary care children's hospital between February 2014 and February 2015 were tested for HAdV by RP v1.7. If the RP v1.7 results were negative for HAdV, then the specimens were reflexed to a HAdV-specific laboratory-developed PCR (LD-PCR) assay for confirmation. A subset of specimens underwent secondary confirmatory testing using another commercially available HAdV PCR assay and a molecular typing assay for species identification. Among 4,750 specimens, a total of 146 specimens (3.1%) were HAdV positive by RP v1.7. HAdV was detected by LD-PCR in an additional 220 specimens that were negative by RP v1.7. Overall, a nearly 5% increase in HAdV detection was observed when RP v1.7-negative specimens were reflexed to LD-PCR testing. RP v1.7 did not detect HAdV with either low viral burden (threshold cycle values of >30) or nonrespiratory species (species A, D, and F), as shown in both clinical and analytic data. While the level of sensitivity of RP v1.7 may be adequate for testing among otherwise healthy children, the decreased sensitivity may be problematic for immunocompromised patients, in whom low levels of HAdV in the respiratory tract may precede systemic infection and require early intervention.

Human adenovirus (HAdV) respiratory infections are associated with 7 to 8% of all identified viral causes of acute respiratory illnesses, especially among children under 5 years of age (1, 2). To date, over 50 HAdV serotypes have been identified; they are divided into 7 species (species A to G) and cause a broad spectrum of clinical diseases, due to the organ tropisms of different species. Species A is primarily associated with respiratory and gastrointestinal (GI) infections, species B, C, and E with respiratory infections, species D with ocular and GI infections, and species F and G with GI infections (3, 4). Additionally, HAdV, especially species C, is known to persist for years in human lymphoid tissue (5–7) and may reactivate and replicate under certain conditions (4, 8).

Clinical interpretation of results for HAdV detection in the nasopharynx (NP) is complicated, in that increased sensitivity is needed in certain populations but increased specificity may be needed in others. For example, even low levels of NP detection in immunocompromised hosts may warrant close monitoring and further intervention (9), as HAdV infections can progress rapidly and cause significant morbidity and mortality in this population (3, 4). In contrast, detection of HAdV in otherwise healthy children may not always indicate disease, due to the propensity of HAdV for prolonged shedding and/or persistence in the tonsils and/or adenoid tissue (10). This is evidenced by the finding that 3 to 11% of asymptomatic healthy children have PCR-detectable HAdV in the NP (11–13).

Amplified molecular testing, such as PCR, has become the standard of care for the diagnosis of respiratory HAdV infections in many laboratories, and a number of respiratory panels for multiplex amplification have been developed. These comprehensive panels can provide point-of-care testing results with a simplified enhanced workflow, compared with laboratory-developed PCR

(LD-PCR). One such panel, the BioFire FilmArray Respiratory Panel (RP) (BioFire Diagnostics, Salt Lake City, UT), detects 17 viral and 3 bacterial targets (including HAdV) from NP swab specimens. Initial reports of lower sensitivity for HAdV in the first commercial release of the product (RP v1.6; sensitivity of 67%) led to an updated version, RP v1.7, which demonstrated improved sensitivity (91%) for the detection of HAdV, mainly due to broader serotype coverage in RP v1.7 (14). However, the true clinical performance of RP v1.7 for HAdV detection in a large pediatric cohort has not been well described.

In this study, we sought to evaluate the clinical performance of RP v1.7 for detection of HAdV in a large pediatric cohort. We compared performance parameters to those for our LD-PCR. In addition, we sought to determine the relative viral burdens and genotypes of HAdV detected, to determine the significant differ-

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FIG 1 Schematic flowchart for patient enrollment, classification, and testing. Specimens testing negative for HAdV by RP v1.7 were all reflexed to LD-PCR testing, and an additional 220 unique specimens were positive by LD-PCR. A subset of randomly selected specimens in the RP (–)/LD-PCR (+) group were tested with a secondary confirmatory test (ProAdeno PCR assay) to compare the clinical performance of our LD-PCR assay. All available HAdV-positive specimens were tested for molecular typing. *, 6 bronchoalveolar lavage fluid or tracheal aspirate samples and 12 duplicate samples. HAdV, human adenovirus; LD-PCR, laboratory-developed PCR; RP, FilmArray Respiratory Panel v1.7.

ences between the assays, and we correlated HAdV characteristics with clinical presentations in children.

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MATERIALS AND METHODS

Study population. Patients with HAdV-positive respiratory specimens, as determined by RP v1.7, that had been collected from the emergency department, urgent care center, or inpatient units of Nationwide Children's Hospital (NCH) between February 2014 and February 2015, were identified using microbiology laboratory records. As a part of the standard of care in our facility, all specimens that were negative for HAdV by RP v1.7 were reflexed to qualitative LD-PCR testing for HAdV. Additionally, a subset of specimens that were HAdV positive by RP v1.7 were also reflexed to LD-PCR testing. Only the first available upper respiratory tract specimen from each patient was analyzed in cases with multiple HAdV-positive specimens collected during the study period. Lower respiratory tract specimens (e.g., bronchoalveolar lavage [BAL] fluid and tracheal aspirate samples) were excluded from analysis, as RP v1.7 has been approved for use only with NP swabs. The use of throat swabs is off-label and was verified by our laboratory; results were included in the analyses. Patients with an underlying medical condition (e.g., prematurity, asthma, congenital heart disease, neurological disorder, or genetic disease) or an immunocompromised condition (e.g., solid organ transplant recipients, patients receiving chemotherapy for an underlying malignancy, or patients receiving immunosuppressive therapy) were included in the final analysis. Medical records were retrospectively reviewed to collect demographic and clinical data. The study was approved by the NCH institutional review board.

FilmArray RP v1.7. RP v1.7 includes assays for the detection of HAdV, coronaviruses (HKU1, NL63, 229E, and OC43), human metapneumovirus (hMPV), influenza A and B viruses (with specific detection of influenza A virus subtypes H1, H1-2009, and H3), parainfluenza virus (PIV) types 1 to 4, respiratory syncytial virus (RSV), human rhinovirus (HRV)/enterovirus (EV), *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*,

and *Bordetella pertussis*. Testing was performed as recommended by the manufacturer (15).

LD-PCR assay. The HAdV-specific real-time PCR assay was based on the protocol developed by Heim et al. (16), targeting a conserved region of the HAdV hexon gene and designed to detect all serotypes of HAdV, as described previously (17). Samples were considered HAdV positive if threshold cycle (C_T) values were ≤ 40 .

Direct HAdV molecular typing. Frozen samples were retrieved to determine HAdV species A to F by molecular genotyping of HAdV directly from respiratory specimens (Fig. 1), using six different primer/probe sets. Primer/probe sets that detect species A, C and D were as described previously, with modification (18); primer/probe sets that detect species B, E, and F were designed on the basis of the available HAdV DNA sequence information (National Center for Biotechnology Information database) (see Table S1 in the supplemental material). Reference strains of 17 HAdV serotypes and 9 clinical isolates with known genotypes were used as positive controls. The primer/probe sets are specific for genotypes with the following exception: the type B primer/probe set detects both type B and type E (19).

ProAdeno PCR assay. The ProAdeno+ assay (ProAdeno PCR assay; Hologic/Gen-Probe, San Diego, CA) is an FDA-cleared, singleplex, real-time PCR assay. For a subset of specimens with discordant results (i.e., RP v1.7 negative but LD-PCR positive), additional testing using ProAdeno kits was performed to compare the clinical performance of our LD-PCR assay to that of the ProAdeno PCR assay. The specimens were either residual nucleic acid eluates used for the LD-PCR assay (extracted on the bioMérieux easyMAG system) that had been stored at -20°C or original specimens that had been stored at -80°C and were extracted on the bioMérieux easyMAG system. Samples represented every month during the 13-month study period except June 2014. A total of 5 μl of specimen extract was then added to 20 μl of each ProAdeno master mix and amplification was performed using a SmartCycler system (Cepheid, Sunnyvale, CA), under conditions specified by the manufacturer.

Comparative limit-of-detection studies. HAdV serotypes 18, 31, 3, 7, 5, 6, 4, 40, and 41, representing species A, B, C, D, E, and F, were purchased

TABLE 1 Comparison of clinical and virological characteristics of patients with HAdV infections in the RP(+) and RP(-)/LD-PCR(+) groups

Characteristic ^a	RP(+) group (<i>n</i> = 146)	RP(-)/LD-PCR(+) group (<i>n</i> = 220)	<i>P</i>
Clinical characteristics			
Age (median [IQR]) (yr)	1.8 (0.95–3.9)	1.5 (0.7–3.5)	NS
Female/male	1/1.5	1/1.1	NS
Underlying condition (no. [%])	66 (45)	100 (45)	NS
Immunocompromised host (no. [%])	7 (5)	16 (7)	NS
Patient location (no. [%])			
Outpatient	32 (22)	15 (7)	
Floor only	78 (54)	121 (55)	<0.0001
PICU	36 (24)	84 (38)	
Length of hospital stay (median [IQR]) (days)	2.8 (1.9–4.5)	2.9 (1.7–4.8)	NS
Fever (>100.4°F) at time of initial evaluation (no. [%])	99 (68)	132 (60)	NS
Clinical phenotype (no. [%])			
Fever alone	19 (15)	24 (11)	
URTI	48 (31)	49 (22)	
LRTI	65 (45)	133 (60)	0.0279
GI disease	9 (6)	10 (5)	
Other	5 (3)	4 (2)	
Coinfection	75 (51)	157 (71)	<0.0001
Virological characteristics			
HAdV <i>C_T</i> (median [IQR])	26.3 (23.2–29.3)	37.4 (35.6–38.8)	<0.0001
Species identification tested (no.)	122	189	
Respiratory species (no. [%])	107 (88) ^b	83 (44) ^b	
Species C	62	76	<0.0001 ^b
Species B/E	45	7	
Nonrespiratory species (no. [%])	1 (1) ^b	34 (18) ^b	
Species A	0	4	
Species D	1	4	
Species F	0	26	
≥2 species ^c	11	5	
Undetermined	3	67	

^a IQR, interquartile range; NS, not statistically significant; PICU, pediatric intensive care unit; URTI, upper respiratory tract infection; LRTI, lower respiratory tract infection; GI, gastrointestinal.

^b The chi-square test was used for proportions.

^c Including 6 specimens with species B/E plus species C, 2 specimens with species C plus species F, 1 specimen with species A plus species C, and 2 specimens with species A plus species D in the RP(+) group and 4 specimens with species C plus species F and 1 specimen with species A plus species F in the RP(-)/LD-PCR(+) group.

from the ATCC (Manassas, VA). Adenoviruses were cultured in RMix-Too shell vials (Quidel, San Diego, CA), with incubation at 37°C for 48 h. The culture supernatants were collected and stored at -80°C as viral stocks. These working stocks were serially 10-fold diluted and were tested in triplicate by RP v1.7, to determine the relative limit of detection (rLOD) for each serotype. The limit of detection (LOD) of each serotype was defined as the lowest concentration that achieved 95% positivity. We were able to perform only a limited number of runs; therefore, the LOD of each serotype is represented as the rLOD, as a range in which the 95% positivity rate fell (see Table 3). The highest 10-fold dilution that tested negative with RP v1.7 for each serotype was also tested by the LD-PCR and ProAdeno PCR assays (see Table 4), to compare the relative sensitivity values for these assays.

Viral load quantification. The viral load for each species was determined as described previously (20). Briefly, a two-reaction system containing six different primer/probe sets was used, with one reaction detecting and quantifying HAdV species A, C, and F and one detecting and quantifying HAdV species B, D, and E. The PCRs were set up in a total of 25 µl, including 5 µl template DNA and 12.5 µl TaqMan Universal master mix (Applied Biosystems, Foster City, CA). The concentrations of primers and probes and the cycling conditions were as described previously (20), and an ABI 7500 thermocycler was used. External standard curves for each reaction were established by preparing serial dilutions of quantified viruses, i.e., HAdV species C (Qnos-tics, Glasgow, Scotland) and HAdV species B (AcroMetrix, Benicia,

CA). Quantitative results were expressed as the number of virus copies per milliliter.

Case classification. Patients were classified into 2 groups, based on the laboratory results of HAdV testing (Fig. 1). For the RP(+) group, HAdV was detected by RP v1.7; for the RP(-)/LD-PCR(+) group, HAdV was not detected by RP v1.7 and was detected only by LD-PCR. Coinfection was defined as detection of a respiratory virus or bacteria using RP v1.7, and findings were available for all specimens.

Clinical phenotypes. Patients were categorized into 5 clinical phenotypes based on their primary symptoms at the time of testing for HAdV, as follows: (i) fever alone with no other specific signs or symptoms; (ii) upper respiratory tract illness (URTI), i.e., upper respiratory tract symptoms such as cough, coryza, conjunctivitis, pharyngitis, and/or pharyngoconjunctival fever; (iii) lower respiratory tract illness (LRTI), i.e., acute respiratory illness with symptoms or signs of lower airway involvement (e.g., dyspnea or tachypnea, hypoxia, and/or abnormal lung examination results); (iv) gastrointestinal (GI) illness, i.e., acute gastroenteritis (AGE) (only if diarrhea was a predominant complaint); (v) other, i.e., the medical documentation was insufficient to classify the illness phenotype or the patient did not meet any of aforementioned criteria for the 4 most common classic presentations of HAdV infections.

Statistical analysis. Mann-Whitney or Kruskal-Wallis tests were used for comparisons between two or several groups as appropriate, and the chi-square test was used for analyses of proportions. Two-tailed *P* values

FIG 2 Comparison of HAdV C_T values in the RP(+) group versus the RP(-)/LD-PCR(+) group. The median HAdV C_T value for 88 available specimens in the RP(+) group was significantly lower than that for all specimens in the RP(-)/LD-PCR(+) group (C_T values of 26.3 [IQR, 23.2 to 29.3] versus 37.4 [IQR, 35.6 to 38.8]; $P < 0.0001$). Three immunocompetent patients in the RP(-)/LD-PCR(+) group with low C_T values are further described in Results.

of <0.05 were considered significant. All tests were performed using GraphPad Prism (San Diego, CA).

RESULTS

Case classification. A total of 4,750 respiratory specimens were tested by RP v1.7, and HAdV was detected in 153 specimens (Fig. 1). Specimens with negative HAdV results by RP v1.7 were all reflexed to LD-PCR testing, according to the standard of care, and HAdV was detected in 231 additional specimens. Overall, 146 specimens from unique patients were classified into the RP(+) group; 220 additional unique specimens that were negative by RP v1.7 and positive by LD-PCR were classified into the RP(-)/LD-PCR(+) group after the exclusion of duplicate samples and lower respiratory tract specimens. We identified 366 specimens (99% NP specimens and 1% throat specimens) for analysis.

Clinical characteristics of groups. The clinical characteristics of each group are presented in Table 1. The median age was 1.6 years (interquartile range [IQR], 0.8 to 3.5 years), and 98% of the patients were <18 years of age. Among the 366 unique patients, coinfection with another virus or bacteria detected by RP v1.7 was common (63%) and was seen more frequently in the RP(-)/LD-PCR(+) group. HRV/EV was the most common viral coinfection (43%), followed by RSV (23%), PIVs (10%), hMPV (8%), coronaviruses (5%), and influenza A and B viruses (5%). Bacterial organisms were codetected by RP v1.7 in 3 specimens (with *M. pneumoniae* in 1 specimen and with *B. pertussis* in 2 specimens).

Virological characteristics of groups. In addition to reflexing of all RP(-) specimens to LD-PCR testing, a subset of RP(+) group specimens ($n = 88$) were also reflexed to LD-PCR testing. All 88 specimens were also positive by LD-PCR. There was a significant difference in median HAdV C_T values between the 2 groups [RP(+) group, median C_T of 26.3 [IQR, 23.2 to 29.3]; RP(-)/LD-PCR(+) group, median C_T of 37.4 [IQR, 35.6 to 38.8]; $P < 0.0001$] (Fig. 2). There were three samples from immunocompetent patients with HAdV C_T values of <30 by LD-PCR among the NP specimens that were missed by RP v1.7 (Fig. 2), as follows: patient 1 (3-year-old female with LRTI with species B/E), C_T of 21.1; patient 2 (6-month-old male with AGE with species A), C_T of 23.7; patient 3 (18-month-old male with LRTI with two HAdV species, i.e., species C and F), C_T of 25.4. Rates of HAdV detection by RP v1.7 were significantly decreased in specimens

with relatively higher LD-PCR C_T values, compared with those with low C_T values (C_T values of ≤ 30 , 95%; C_T values of 30 to 40, 6%; $P < 0.0001$) (Fig. 3).

A total of 311 specimens were tested by direct molecular typing, and species were identified in 77% of the specimens (Table 1). Species C was the most common species (48%) in both groups, followed by species B/E (19%); species B/E was more frequently detected in the RP(+) group (37% versus 4%; $P < 0.0001$). Sole infections with species A, D, and F were more frequent in the RP(-)/LD-PCR(+) group ($P < 0.0001$). Among the 88 specimens in the RP(+) group that were reflexed to LD-PCR testing, 73 were available for typing, and the majority (95%) were successfully typed. In the RP(-)/LD-PCR(+) group, 189 specimens were available for typing, and 65% of those were successfully typed. The HAdV species in each group in relation to the LD-PCR C_T values are presented in Fig. 3.

Concordance of LD-PCR assay results with ProAdeno PCR assay and direct molecular typing results in the RP(-)/LD-PCR(+) group. We evaluated a subset of randomly selected RP v1.7-negative specimens ($n = 81$) and further tested them with a FDA-cleared qualitative assay, the ProAdeno PCR assay. Of those 81 specimens, 55 (68%) were positive for HAdV by the ProAdeno PCR assay (Table 2). In addition, typing analysis was performed for the 81 specimens, and 61 specimens (75%) were typed successfully. Altogether, findings for 68 (84%) of 81 RP(-)/LD-PCR(+) specimens were confirmed by a second assay. As expected, less agreement was observed at high LD-PCR C_T values (C_T values of 35 to 40), due to the difference in the sensitivities of the assays, as described below.

Limits of detection. Tables 3 and 4 show the rLODs and comparative sensitivities for RP v1.7. Consistent with the description in the assay instructions (15), RP v1.7 has relatively high rLODs (i.e., low sensitivity) for species A, D, and F, while the rLODs for species B, C, and E are relatively low (high sensitivity) (Table 3). Comparative sensitivities for RP v1.7 and the LD-PCR and ProAdeno PCR assays are shown in Table 4. The concentration used for testing was one that represented the highest viral load that RP v1.7 was unable to detect. As seen in Table 4, the LD-PCR and ProAdeno assays had different sensitivities depending on the species and/or serotypes tested, but both were more sensitive than RP v1.7 in most instances.

DISCUSSION

We demonstrated in this study that RP v1.7 revealed a prevalence of HAdV of 3.1%, compared with a prevalence of 7.7% detected by adding the LD-PCR assay, in this large pediatric cohort. HAdV has previously been reported to be associated with 7 to 8% of pediatric respiratory infections (1, 2), which is closer to the prevalence in this cohort demonstrated using LD-PCR. Although the sensitivity of RP v1.7 has improved, compared to the earlier version RP v1.6 (14), an additional 4.6% of specimens ($n = 220$) were detected only by LD-PCR. This was mainly due to the inability of RP v1.7 to detect HAdV at low viral burden (only 6% of specimens with LD-PCR C_T values of 30 to 40 were detected). In addition, we observed that the sensitivity of RP v1.7 for species C in clinical specimens was lower than that of LD-PCR, as $>50\%$ of species C specimens were missed by RP v1.7. This increase in sensitivity of LD-PCR was confirmed with two other PCR assays, i.e., the ProAdeno PCR and direct molecular typing PCR assays.

The analytical sensitivity performance characteristics of the RP

FIG 3 Rates of human adenovirus detection by RP v1.7 according to LD-PCR C_T values. Rates of HAdV detection by RP v1.7 were significantly decreased in specimens with relatively higher LD-PCR C_T values, compared with those with low C_T values (C_T values of ≤ 30 , 95%; C_T values of 30 to 40, 6%; $P < 0.0001$). RP, FilmArray Respiratory Panel v1.7; LD-PCR, laboratory-developed PCR; HAdV, human adenovirus.

v1.7 and LD-PCR assays support these findings. To analytically evaluate the comparative LODs for each species, we performed a multireaction quantitative PCR. The assay uses 2 separate reactions with multiple targets, i.e., 1 reaction for species A, C, and F and 1 reaction for species B, D, and E. Quantitation was defined on the basis of the highest viral load between the 2 reactions; of note, it was rare to observe cross-reactions leading to amplification in both reactions. Other investigators have used single-target/single-reaction PCR assays to quantitate HAdV. That method may be inaccurate, however, due to the species-related differences in amplification efficiency, leading to inexact quantification. To our knowledge, this is the first time that the LODs of RP v1.7 have been addressed specifically for each species with viral loads being defined by a two-reaction system. Our findings confirmed the manufacturer's data, based on 50% tissue culture infective dose (TCID₅₀) values (15), that RP v1.7 detects species A and F (serotypes 31, 18, 40, and 41) with very low sensitivity; viral loads of $>1 \times 10^6$ copies/ml were required for detection by RP v1.7 (Table 4). We also observed differences in sensitivity within species (e.g., species F, serotype 40 versus serotype 41). These findings are

largely caused by the high levels of genomic heterogeneity across the HAdV family, as well as within the same species. The analytical sensitivity of RP v1.7 for species B and C, represented by serotypes 3, 7, 5, and 6 (those most commonly seen and associated with respiratory infections in children), is close to that of LD-PCR, with a rLOD of <500 copies/ml. As a limitation of this study, we were not able to evaluate the rLODs of RP v1.7 for other serotypes in species B and C. Similar to our findings for species F, however, different LODs within a species are expected. As seen in the RP(-)/LD-PCR(+) group (Fig. 2), there were three samples with high viral burdens that were missed using RP v1.7. One was a specimen with species A, which was likely missed due to the low sensitivity of RP v1.7 for that species. Findings for the other two samples (one sample with species B/E and the other with codetection of species C and F) may have been due to different LODs within those species. For the latter 2 specimens, the ProAdeno PCR assay confirmed the LD-PCR results; therefore, we do not think that the findings were due to technical errors. These data suggest that, rather than inherent decreased sensitivity due to the multiplex nature of RP v1.7, the lack of species coverage (species

TABLE 2 Concordance of LD-PCR results with ProAdeno PCR and direct molecular typing assay results for the RP(-)/LD-PCR(+) group

PCR method with positive result	No. with HAdV C_T by LD-PCR of ^a :				Total no. detected (n = 81)	Agreement with LD-PCR (%)
	20 to <25 (n = 1)	25 to <30 (n = 1)	30 to <35 (n = 25)	35 to 40 (n = 54)		
ProAdeno assay	1	1	21	32	55	68
Direct molecular typing assay	1	1	23	36	61	75
ProAdeno assay or direct molecular typing assay	1	1	25	41	68	84

^a All specimens were positive by LD-PCR, with C_T values in the ranges shown. The number of positive specimens determined by other methods is shown in each row.

TABLE 3 Species-specific relative limits of detection of RP v1.7 for ATCC strains

Species and serotype	Relative LOD (log ₁₀ copies/ml) ^a	
	Lower limit	Upper limit
A		
18	6.0	7.0
31	7.0	8.0
B		
3	<2.71	
7	<2.71	
C		
5	<2.71	
6	<2.71	
D		
13	3.0	4.2
18	3.0	3.8
E		
4	2.6	3.4
F		
40	6.5	7.5
41	4.4	5.3

^a The LOD of each species is presented as a viral load range in which the 95% positivity rate fell. We were unable to determine the lower limit for the serotypes tested in species B and C.

A, D, and F) and heterogeneity within a given species (specifically, species C) were responsible for the decreased sensitivity for HAdV.

Interpretation of HAdV detection in pediatric respiratory samples can be difficult, as HAdV (especially species C) is known to establish persistence, with low levels of detection in tonsils and adenoids for years (5, 6). Therefore, detection of HAdV in the NP by PCR does not necessarily reflect acute disease in children (10). A delicate balance between sensitivity and specificity is needed for optimal predictive values for an assay for HAdV-related disease. An important question is whether the RP v1.7(-)/LD-PCR(+) specimens were clinically relevant as the cause of acute illness. Since there is no established gold standard method to diagnose acute HAdV-related disease and we were unable to perform a longitudinal study, we have utilized recovery of HAdV from cultures as a marker of active disease in our previous study (19). Others have demonstrated that there is a much lower rate of asymptomatic detection among children using culture (0.6%) versus PCR (3 to 11%) (1, 11–13). On the basis of those analyses, we think that, using our LD-PCR assay, C_T values of <36 are generally relevant (because they are highly correlated with positive HAdV results from viral cultures). Of the RP v1.7(-)/LD-PCR(+) specimens, 60 (26.2%) were under that threshold.

For immunocompetent hosts with a low risk of disseminated systemic infection, an assay with low sensitivity for the molecular detection of HAdV may be adequate, as low levels of HAdV during shedding would be less likely to be identified, which might prevent confusion and possible misattribution of illness to HAdV (17). The majority of our cohort was immunocompetent (94%), and patients in the RP v1.7(+) group had significantly higher viral loads (C_T values of 26.3 versus 37.4; $P < 0.0001$) and more URTI,

TABLE 4 Comparison of sensitivities of RP v1.7 versus LD-PCR and ProAdeno PCR assays for ATCC strains

Species and serotype	Viral load (log ₁₀ copies/ml) ^a	RP v1.7 result	LD-PCR C_T	ProAdeno C_T
A				
18	6.0	Neg ^b	21.3	28.2
31	7.0	Neg	17.4	16.2
B				
13	1.0	Neg	42.7	ND
7	1.0	Neg	ND	ND
C				
5	1.0	Neg	41.0	ND
6	1.6	Neg	39.6	36.2
D				
3	2.3	Neg	40.2	36.5
18	3	Neg	37.1	34.1
E				
4	2.6	Neg	38.3	40.3
F				
40	6.5	Neg	19.5	22.3
41	3.4	Neg	34.0	32.1

^a Comparative testing was performed using the highest viral loads tested that were negative by RP v1.7.

^b Neg, negative; ND, not detected.

representing acute HAdV infections. Conversely, those in the RP(-)/LD-PCR(+) group were more ill, in that they tended to have more LRTI and were more likely to be inpatients. We also noted a higher coinfection rate in the latter group. Although low levels of HAdV detection could have been due to prodromal or late illness, we think that some of the patients had incidental detection of HAdV, as we described previously (17, 21). Similarly, among 483 respiratory specimens from immunocompetent pediatric patients in our previously analyzed cohort, 52% were culture positive for HAdV and had significantly higher viral burden, compared to those with no growth in culture (culture-positive specimens, median C_T of 25.8 [IQR, 22.5 to 29.9]; culture-negative specimens, median C_T of 37.4 [IQR, 35.2 to 38.8]; $P < 0.0001$) (19). Although the studies were performed with two different cohorts, the similar performance characteristics of viral culture and RP v1.7 suggest that RP v1.7 is useful for the diagnosis of acute HAdV-related disease in immunocompetent individuals. As a result of our analyses, we have discontinued routine reflex testing of RP v1.7-negative samples for immunocompetent outpatients.

In contrast to immunocompetent hosts, the use of a less sensitive assay for immunocompromised children, who are at higher risk of disseminated illness, could be detrimental and possibly lead to adverse outcomes, as it could delay appropriate intervention and therapy. It has been reported that early detection of HAdV in the NP after allogeneic hematopoietic stem cell transplantation (HSCT) may precede early disseminated HAdV infection (i.e., detection of HAdV DNA from ≥ 2 organ systems) and impending progression to fatal end-organ disease (9, 22). Some centers monitor NP specimens serially for HAdV detection for the prediction of HAdV-related disease and institute preemptive antiviral treatment (23). While the RP v1.7 assay was designed to target respi-

ratory HAdV species (species B, C, and E), it is important to note that virtually any species, including species A, D, and F, can cause fulminant HAdV-related disease in immunocompromised hosts, including allogeneic HSCT recipients (3, 4, 24). As an example, during the study period, we identified a severely immunocompromised patient who had undergone HSCT for whom RP v1.7 failed to detect HAdV in respiratory specimens; HAdV was detected by our reflex LD-PCR protocol. The patient had progressive disease, with detection of HAdV in blood, NP, BAL fluid, urine, and stool specimens, and developed lower respiratory tract symptoms. The NP specimen was negative by RP v1.7 and positive by LD-PCR, with an estimated viral load of 2.5×10^5 copies/ml (C_T of 31.2), and molecular typing revealed species A. Therefore, respiratory HAdV infection could have been missed in this severely immunocompromised patient if RP v1.7 had been the only testing method used for the diagnosis of HAdV infection. Based on this experience, we discourage the use of RP v1.7 and suggest the use of the LD-PCR assay for the diagnosis of HAdV infection in this patient population in our hospital. Although the assay instructions state that RP v1.7 can detect some nonrespiratory serotypes, including species A (15), our comparative LOD testing demonstrated that only high viral loads ($>1 \times 10^6$ copies/ml) could be detected. Also, from an epidemiology and infection control standpoint, the sensitivity of an assay can affect isolation practices. In the case of highly vulnerable immunocompromised patients, use of a more sensitive assay seems prudent to prevent nosocomial transmission (22, 24).

There are some limitations of our work. The LD-PCR assay used for clinical testing was semiquantitative. The specific C_T values calculated with our laboratory assay are not directly equivalent to those obtained with other PCR assays. However, we demonstrated the differences in analytical sensitivity of the RP v1.7 and LD-PCR assays using a quantitative assay. Although we did not test all serotypes within each species, we were able to perform LOD studies with some representative HAdV serotypes. As with all molecular assays, contamination is a great concern. We tried to address the possibility that the increased sensitivity of our LD-PCR assay was attributable to laboratory contamination by confirming the results with secondary testing. For a subset of specimens, testing was repeated with the original specimens to confirm the results, and there was 100% concordance with secondary testing results (data not shown). Additionally, the LOD analyses support the increased sensitivity of LD-PCR over RP v1.7. In the absence of a longitudinal study, it is difficult to identify the significance of low levels of HAdV detected in respiratory specimens from acutely ill children.

Multiplex PCR assays for respiratory viral testing are quickly becoming the standard of care for the diagnosis of respiratory illnesses in children. RP v1.7 is a highly automated assay that provides comprehensive panels with a rapid turnaround time, which is beneficial for both clinicians and patients (14, 25). As shown in our data, RP v1.7 was able to detect the majority of HAdV respiratory species (i.e., species B, C, and E) in clinical specimens when the viral burden was high, and it is likely appropriate for testing in outpatient and/or immunocompetent populations. However, our data also suggest that it is imperative that clinicians be aware of the limitations in sensitivity of RP v1.7 for HAdV detection among immunocompromised patients. A negative result for HAdV with RP v1.7 should be interpreted cautiously, as the negative predictive value may be compromised, and additional testing using an

assay with greater sensitivity to detect HAdV should be considered for immunocompromised patients.

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