Evaluation of a Rapid Quantitative Diagnostic Test for Adenovirus Type 4

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Acute respiratory disease (ARD) due to adenoviruses is a reemerging disease in military recruits. It is a challenge for clinicians to accurately diagnose this disease and to appropriately treat affected individuals. This study investigated the utility of a quantitative, rapid-cycle, real-time fluorogenic polymerase chain reaction (PCR) technique for detecting adenovirus type 4 (Ad4) in a clinical setting. Throat swab specimens and clinical data were collected from US Army basic trainees hospitalized with ARD at Fort Jackson, South Carolina. A total of 140 throat swab specimens were collected from 83 subjects. Rapid PCR results (obtained in <2 h) had a sensitivity of 100% and a specificity of 100%, compared with viral culture. There was no difference, qualitative or quantitative, between frozen and fresh samples for PCR detection of Ad4. Individuals with test results positive for Ad4 were hospitalized longer than were individuals with negative test results. Higher virus loads at hospital admission corresponded to longer lengths of stay for Ad4-positive subjects.

Adenoviruses (Ads) have been associated with acute respiratory disease (ARD) in military recruits since 1953 [1]. Epidemiological studies conducted during World War II [2] and during the 1950s and 1960s [3–6] reported the profound impact of Ad-associated ARD on military trainees. Almost all recruits had upper respiratory symptoms, as many as one-half sought medical care, and as many as 20% were hospitalized. One in 10 military recruits with Ad-associated ARD had atypical pneumonia [2, 3]. Ad caused 90% of cases of pneumonia in recruits. Live, enteric-coated, oral vaccines for the predominate Ad types 4 and 7 were developed, and routine administration of these vaccines, beginning in 1971, successfully controlled Ad-associated ARD [7–9]. Ad vaccine production was halted in 1996, and the last supplies were exhausted in early 1999. Postvaccine experience with Ad-associated ARD in military trainees has mirrored that of the time before routine administration of the Ad vaccine was instituted [10, 11].

Ad-associated disease has been reported in civilian populations. Sporadic outbreaks have occurred among immunocompetent populations at long-term care facilities, day care facilities, a Job Corps training camp, and in immunosuppressed populations (e.g., bone marrow transplant and organ transplant recipients) [12–17].

With the loss of access to the Ad vaccines, it is a challenge for clinicians to accurately diagnose Ad-associated ARD and to appropriately treat the large
numbers of affected individuals. Distinguishing on clinical grounds between ARD with or without pneumonia caused by a bacterial agent, by influenza, or by an Ad may be impossible. Current testing modalities are labor- and time-intensive, limited in sensitivity, specificity, and availability, and restricted to identifying only a few potential agents [6, 18].

A quantitative, rapid-cycle, real-time fluorogenic PCR technique for detecting Ad type 4 (Ad4) in freshly obtained patient specimens was developed at the Walter Reed Army Institute of Research (WRAIR; Silver Spring, MD) [19]. Compared with viral culture, this system had 98% sensitivity and 100% specificity when used on the TaqMan 7700 (Perkin Elmer) (PCR1) in the research laboratory [19]. This PCR system was modified for a small, portable unit (PCR2), the Smart Cycler TD system (Cepheid), for possible use in clinical settings where rapid evaluation of respiratory disease specimen would be beneficial (e.g., field settings, outbreak sites, and established clinical laboratories).

We studied throat swab specimens from US Army basic trainees hospitalized with ARD at Fort Jackson, South Carolina, for Ad4 by means of PCR2. We compared the results from PCR2 with results from traditional cell culture virus isolation tests and results obtained by means of PCR1. The results from frozen and thawed specimens were compared with those from fresh specimens. Additionally, we collected clinical case data on soldiers hospitalized with ARD and evaluated the relationship between virus load (as determined by PCR2) and clinical course.

**MATERIALS AND METHODS**

*Case definition and subjects.* Potential volunteers were US Army recruits, aged ≥18 years, participating in 8 weeks of basic training at Fort Jackson. Subjects with ARD were recruited from trainees admitted to Moncrief Army Community Hospital (MACH; Fort Jackson) over a 5-week period. ARD was defined as an oral temperature of ≥38.1°C at presentation and the presence of cough or sore throat. By US Army Medical Command policy, all basic trainees seen at a clinic who had a temperature of ≥38.1°F were admitted to the hospital. To focus on the agent initially causing illness, only patients with fever of <72 h duration were studied. Patients admitted on weekends were excluded from the study. Potential subjects were informed about the study and asked to review and sign the consent form. Of 210 patients admitted to MACH with ARD during the study period, 95 eligible patients (45.2%) were asked to participate, and 87.4% of these volunteered (83 subjects). Data were not collected for nonparticipants.

All protocols were approved by the Institutional Review Board of the Walter Reed Army Institute of Research, the Human Subjects Research Review Board of the Office of the Surgeon General of the Army, and the Human Use Committee at Eisenhower Army Medical Center (Fort Gordon, GA), which supports MACH.

*Data collection and samples.* Volunteers completed a form that requested demographic data (age, sex, military unit, and week of basic training) and clinical data (days of cough or sore throat and history of fever). Related information was obtained from medical records. Throat swab specimens were collected within 24 h after admission and every hospital day thereafter. Cotton-tipped applicators were used to swab the tonsillar pillars and posterior pharynx. The swabs were sterilely broken off into viral culture media [10] and transported to the MACH clinical laboratory. Samples were collected in November and December 2001 and January 2002, historically a time of peak Ad4 activity at Fort Jackson. During the first 30 days of the study, PCR2 analyses were performed on unfrozen, fresh aliquots in the MACH clinical laboratory. Less than 2 h passed from the time a sample was brought to the MACH laboratory to acquisition of the final PCR result. Clinical and laboratory personnel were blinded to both patient identification and PCR results.

On receipt, viral culture media were split into aliquots for PCR analyses and cell culture. All throat swab specimens were frozen for shipment to WRAIR for further analyses and virus isolation. Subjects enrolled during the first 30 days of the study were asked to donate acute-phase and convalescent-phase serum. Blood samples were collected on the day of enrollment.

![Figure 1](https://academic.oup.com/cid/article-abstract/38/3/391/291434) Figure 1. Quantitative results of adenovirus 4 testing by mobile, multiplex, fluorogenic real-time PCR in fresh and frozen samples. Fresh samples were analyzed at Fort Jackson, SC, within 30 min after collection. Frozen samples were processed at the Walter Reed Army Institute of Research (Silver Spring, MD). Student’s t-test for paired samples found no difference between the log of viral genome copies per mL (log VGC) for fresh and frozen samples (P = .066).
and ~14 days later. Serum was separated and frozen for transport to WRAIR.

**PCR specimen preparation.** The methods used for PCR specimen preparation have been published elsewhere [19]. In brief, an aliquot of viral culture media was diluted 1:5 with 0.1 mM Tris-EDTA pH 8.0 and boiled for 20 min. Ad detection was performed in 25-μL reaction volumes containing 2 μL DNA template, buffer, nucleotides, MgCl₂, forward primer, fluorogenic probe, reverse primer, and polymerase. PCR mixtures were incubated at 50°C for 2 min, then at 95°C for 10 min, followed by 40 cycles of 2-step amplification at 99°C for 15 s and 60°C for 1 min. A 6-point standard curve based on known concentrations of viral genome copies (VGCs) was used in each run along with 2 negative controls. Samples were considered positive for Ad4 by PCR2 if they attained a fluorogenic threshold value of >10 luminescence units in <36 PCR cycles. All samples that attained this threshold before 36 PCR cycles were translated to VGC concentrations via a best-fit line of the standard curve for that run. Qualitative and quantitative data were available on a real-time basis. Samples that underwent ≥36 cycles without generating a signal were considered negative for Ad4. Samples were positive for Ad4 by PCR1 if >700 VGCs/mL were detected; viral detection below this level was not reproducible and was considered a negative result.

**Virus isolation and identification.** Cell culture for Ad isolation was performed in A549 cells with media refreshed weekly for a minimum of 3 weeks and examined for cytopathic effects [10]. Cells from culture were harvested, and, for each patient, a single isolate that developed Ad-type cytopathic effect was identified by neutralization testing with reference antiserum to Ad type 3, type 4, and type 7 [12], the prevalent serotypes among US military populations [6]. Infected cell cultures from isolates that were not identified in neutralization tests with reference antiserum to Ad were studied by electron microscopy [20]. Untyped Ad isolates were identified in neutralization tests with antisera to additional subtypes (e.g., type 21).

**Serum antibody tests.** Anti–Ad4 serum neutralizing antibody titers were determined in A549 cells [21]. Serum specimens were defined as antibody positive if a titer of ≥1:4 was achieved. A significant increase in antibody titer was defined as a ≥4-fold increase between acute-phase and convalescent-phase specimens.

**Data management and statistical analysis.** Data were analyzed by SPSS version 11.0 (SPSS). Comparison of Ad4-positive and -negative subjects, as determined by PCR2, was performed, and relative risks were calculated with 95% CIs for clinical parameters and outcomes. Dichotomous variables were examined with Fisher’s exact test, and continuous variables were studied with Student’s t test (2-tailed). A logistic regression model that used clinical parameters was developed to predict Ad4 status. A logistic model that used virus load was also developed to predict length of hospital stay.

### Table 1. Comparison of adenovirus type 4 detection in both fresh and thawed throat swab specimens by viral culture, laboratory-based fluorogenic PCR, and mobile, multiplex, fluorogenic real-time PCR.

<table>
<thead>
<tr>
<th>Comparison method, PCR method, result</th>
<th>No. of specimens, by result of comparison method</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral culture of thawed specimens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR2 analysis of fresh specimens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>54</td>
<td>100 (93.4–100)</td>
<td>100 (85.2–100)</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR2 analysis of thawed specimens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>95</td>
<td>96.0 (90.0–98.9)</td>
<td>100 (91.4–100)</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>0</td>
<td>41</td>
</tr>
<tr>
<td>PCR2 analysis of fresh specimens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>98</td>
<td>99.0 (94.5–100)</td>
<td>100 (91.4–100)</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>0</td>
<td>41</td>
</tr>
<tr>
<td>PCR2 analysis of thawed specimens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>53</td>
<td>98.2 (93.4–100)</td>
<td>100 (85.2–100)</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>0</td>
<td>23</td>
</tr>
</tbody>
</table>

**NOTE.** PCR1, laboratory-based fluorogenic PCR; PCR2, mobile, multiplex, fluorogenic real-time PCR.

* Specimens collected at Fort Jackson, South Carolina, were stored at −80°C and shipped to the Walter Reed Army Institute of Research, Silver Spring, MD, where they were thawed and processed.

* Specimens collected at Fort Jackson, South Carolina, were tested within 30 min after collection.
RESULTS

General. Sixty-eight (82.0%) of 83 subjects recruited during the 5-week study were men. The average age was 21.5 years (range, 18–34 years). The total Fort Jackson recruit population during the study had an average age of 21.4 years, and 82.0% were men. Subjects were in training weeks 0–8 (recruits in the reception station, awaiting the start of basic training, were in training week 0); the average subject was in training week 4.7. Twenty-nine (34.9%) of the subjects had single-day hospitalizations; 31 (37.3%) had 2-day hospitalizations; 12 (14.5%) had 3-day hospitalizations; 5 (6.0%) had 4-day hospitalizations; and 6 (7.2%) had 5-day hospitalizations. Not every subject had a throat swab specimen collected every day; 140 throat swab specimens were collected from the 83 subjects. Subjects had 1–4 throat swab specimens collected; 25 (30.1%) had 2 specimens, 10 (12.0%) had 3 specimens, and 4 (4.8%) had 4 specimens collected. Acute-phase serum samples were collected from 38 subjects; convalescent-phase samples were collected from 23 (60.5%) of the 38 subjects.

Laboratory results. Of the 140 throat swab specimens tested by viral culture, 104 (74.3%) showed viral cytopathic effects; 99 of these (70.7% of 140) were positive for Ad4. Virus isolates were obtained from 60 (72.3%) of the 83 individuals tested, with positive identification of the viral agent in all cases. Fifty-five (66.3%) of 83 subjects tested positive for Ad4, 2 (2.4%) tested positive for Ad type 3, 1 (1.2%) tested positive for Ad type 21, and 2 (2.4%) tested positive for herpesvirus. The isolates of the 2 herpesviruses and an Ad isolate were identified as type 21 in neutralization tests. The Ad isolate was later identified as type 21 in neutralization tests. Acute-phase serum samples were collected from 23 (60.5%) of the 38 subjects; convalescent-phase samples were collected from 23 (60.5%) of the 38 subjects.

During the first 30 days of the study, 77 specimens were collected and tested by PCR2 as fresh samples. The on-site PCR results for these 77 fresh specimens agreed completely with virus isolation results (sensitivity, 100%; 95% CI, 93.4–100.0; specificity, 100%; 95% CI, 85.2–100.0). The use of PCR2 on paired fresh and frozen samples yielded a qualitative agreement of 98.7%. Quantitative agreement was also excellent, with a linear fit through the origin with a slope of 0.98 and an R² value of 0.87 (figure 1). Analysis of paired fresh and frozen sample results by means of Student’s t test showed no statistical difference in log of the VGC concentration at the 95% confidence level (P = 0.066). Additionally, PCR results for 140 specimens that were frozen, shipped to WRAIR, thawed, and analyzed with PCR2 compared well with culture results (table 1). For the 140 thawed samples, PCR2 had a sensitivity of 96.0% (95% CI, 90.0–98.9) and a specificity of 100% (95% CI, 91.4–100) compared with viral culture (table 1). The 4 false-negative findings by PCR2 were confirmed to be Ad4 by culture, neutralization testing of the isolate, and PCR1; paired serum samples were not available for these subjects. All samples with false-negative results had low virus loads of <100 VGC per reaction (2-μL sample), below the reproducible range of detection for PCR2.

PCR1 detected Ad4 in all frozen and thawed samples identified as positive for Ad4 by PCR2 and in 3 additional samples in which Ad4 was not detected by PCR2 (table 1). Compared with viral culture, PCR1 had a sensitivity of 99.0% (95% CI, 94.5–100.0) and a specificity of 100% (95% CI, 91.4–100.0) (table 1).

For subjects from whom multiple swab specimens were obtained over multiple days (n = 39), those whose specimens tested positive tended to have Ad4 positive specimens throughout their stay, as determined by culture, PCR2, and PCR1. Those whose specimens tested negative at admission tended to continue to have Ad4-negative specimens, with 2 exceptions. One subject was Ad-negative by culture, PCR1, and PCR2 for the first 2 days of hospitalization and became Ad-positive by culture and PCR1 on the last 2 days of hospitalization. Another subject had samples obtained on 2 consecutive days that were both positive for Ad4 by culture, PCR1, and PCR2 and a sample obtained on the third day that was negative for Ad4 by all 3 methods.

Serologic testing. Of the 23 subjects with paired serum samples, 12 showed a ≥4-fold rise in antibody titer. All 12 were positive for Ad4 by viral culture, PCR1, and PCR2. None of the 11 subjects without a 4-fold rise in Ad4 antibody titer were positive for Ad4 by viral culture or PCR.

Outbreak characteristics. The proportion of recruits studied who were positive for Ad4 by PCR2 increased during the 5-week study period, from 50.0% in the first 2 weeks to 91.0% during the fifth week (figure 2). These increases were consistent with observations from ongoing surveillance of the entire train-
Table 2. Relative risk for adenovirus type 4 (Ad4) infection among US Army basic trainees hospitalized with acute respiratory disease.

<table>
<thead>
<tr>
<th>Clinical characteristic</th>
<th>No. of patients</th>
<th>Relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With Ad4 infection</td>
<td>Without Ad4 infection</td>
</tr>
<tr>
<td>Respiratory symptom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sore throat or cough</td>
<td>55</td>
<td>28</td>
</tr>
<tr>
<td>Cough only</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Sore throat only</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Sore throat and cough</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>Duration of respiratory symptoms, days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>≥4</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>38.1–38.3</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>38.4–38.9</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>39.0–39.4</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>39.5–40.0</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>≥40.1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Duration of fever, days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>9</td>
</tr>
<tr>
<td>≥3</td>
<td>19</td>
<td>5</td>
</tr>
</tbody>
</table>

NOTE. All subjects had a minimum temperature of ≥38.1°C and a cough or sore throat. Patients with only a cough and fever were almost 12 times more likely to not have adenovirus infection. 

Clinical correlates. Age, sex, and clinical variables were not predictive of a patient being positive for Ad4 by PCR2 (table 2). Having a cough without a sore throat at presentation was correlated with the absence of Ad4 (relative risk, 11.79; P = .003). Subjects who tested positive for Ad4 tended to have longer hospital stays than did Ad4-negative subjects (2.3 ± 0.33 days vs. 1.8 ± 0.39 days; P = .034). Subjects with virus loads at initial presentation at or greater than the mean observed for all Ad4-positive specimens (log VGC/mL ≥5.2) had longer lengths of stay in the hospital than those subjects with virus loads less than the mean and those subjects who were Ad4-negative (2.7 days vs. 1.9 days and 1.7 days, respectively; figure 3). Conversely, the longer the duration of stay, the more likely that a subject was Ad4 positive. Of the 11 patients hospitalized for ≥4 days, only 1 patient was negative for Ad4. That patient was positive for Ad type 3. For Ad4–positive subjects, the average initial log VGC concentration (±SD) was 5.2 ± 0.3 VGCs/mL (α = 0.05), corresponding to $4 \times 10^{11}$ VGCs per swab specimen. For those subjects with multiple samples, virus loads in the pharynx remained high during the hospital stay and at discharge from the hospital (figure 4).

DISCUSSION

Clinical impact. There is a need for a rapid, accurate diagnostic test for Ad because the etiology for ARD in hospitalized recruits cannot be identified on the basis of clinical signs and symptoms. We found that real-time PCR testing of freshly obtained patient specimens provided results in <2 h and identified patients who were Ad4-positive or -negative. Qualitative and quantitative PCR results were predictive of length of hospital stay. Patients positive for Ad at hospital admission had longer hospitalizations. Those with the highest virus loads, as determined by PCR, had the longest lengths of stay. Our results indicated that the virus loads in the throats of hospitalized ARD patients were high at admission and remained high throughout hospitalization and at discharge. High virus loads in the throats of recruits returning to their units after hospitalization may contribute to the spread of ARD at military training centers. This observation needs more study.

Ad4 is currently responsible for the majority of Ad-associated morbidity among military recruits, but it is not the only agent causing ARD in recruits, and mixed infections may occur. Gray leaking population at Fort Jackson. For the overall training population at Fort Jackson during the study period, the ARD attack rate was 0.58 ARD cases/100 recruit-weeks (range, 0.39–0.80 cases/100 recruit-weeks). Individual companies (each comprising ~200 recruits) had significantly higher ARD attack rates (as many as 8.7 ARD cases/100 recruit-weeks), which had peaked in the fourth and fifth weeks of training.

Figure 3. Average hospital length of stay for subjects negative for adenovirus type 4 (Ad4) by mobile, multiplex, fluorogenic real-time PCR (PCR2) at admission, for subjects in the high viral genome copies (VGCs) group (i.e., those with initial virus loads equal to or greater than the overall average of 5.2 VGCs/mL ± 0.29; α = 0.05), and for subjects in the low VGCs group (i.e., those with lower than average virus loads). Those patients with initial virus loads greater than the average titer had longer lengths of stay (2.7 days ± 0.28; α = 0.05) than those with lower than average virus loads (1.9 days ± 0.14) or those who were Ad4 negative (1.8 ± 0.39 days; α = 0.05) (P = .004). Similar results were obtained from laboratory-based fluorogenic PCR.
et al. [6] identified the need for rapid diagnostic tests for multiple viral and bacterial respiratory disease agents at military training centers. Our assay improves our diagnostic capability for ARD in military recruits and is a first step toward a clinically relevant, multiplex, real-time PCR capable of detecting Ad4 and other significant respiratory pathogens, including Ad type 3, type 7, and type 21. Viral agent identification was achieved for 60 of 83 patients. We did not attempt to identify other viral or bacterial agents known to cause ARD.

Our study population was small but was representative of the Fort Jackson military recruit-trainee population, and the outbreak we studied was typical. We found that Ad4 attack rates peaked in the fourth and fifth weeks of training, which is consistent with previous studies [21, 22]. The overall Fort Jackson attack rate was 0.58 cases/100 recruit-weeks, but individual unit attack rates were as much as 8.7 cases/100 recruit-weeks. This sporadic occurrence of Ad-associated ARD in immunocompetent recruits has been described previously [23].

We used one of several mobile, multiplex, fluorogenic real-time PCR devices currently available (designated PCR2). These exhibit the small size, portability, speed, and ease of sample processing that made PCR2 desirable for this study. Although we found the larger, laboratory-based fluorogenic PCR (PCR1) to be slightly more sensitive, the characteristics and the high sensitivity and specificity we found for PCR2 indicate that portable fluorogenic PCR technology is well suited for outbreak and field investigations and for use in established clinical laboratories.

**Accuracy of fluorogenic PCR.** Compared with viral culture and isolation, PCR2 had a sensitivity of 96.0% and a specificity of 100.0%. PCR1 had greater sensitivity, 99.0%, and a specificity of 100.0%. Samples that were negative for Ad4 by PCR2 and positive by culture had low virus loads. The clinical relevance of this finding is unknown. These individuals could have had Ad4 infections that caused their hospitalizations, or the isolation of Ad4 could have been incidental.

**Fresh and frozen specimens.** There were no significant quantitative or qualitative differences between samples that were analyzed for Ad4 before and after freezing. Therefore, samples may be frozen and stored for PCR analysis of Ad4 at a later time. Similar results may not be observed with other ARD agents.

**Future research.** Fluorogenic, real-time PCR is important because (1) it can detect the nucleic acids of pathogens; (2) it has multichannel capabilities, enabling a single run of a single sample to provide results on multiple pathogens; and (3) it has the capacity to identify specific genetic traits (e.g., virulence and antibiotic resistance) of a pathogen [24]. Because many samples can be assayed for many possible pathogens in a short time, PCR2 and similar systems can be routinely used to identify the cause of and to follow the course of epidemics. The potential uses of PCR2-like systems to identify and monitor multiple pathogens in vaccine trials, other clinical studies, epidemiologic surveillance, and environmental studies to define patterns of Ad transmission and spread deserve consideration.

In conclusion, the findings summarized here confirm and extend our initial findings that highly sensitive and specific diagnosis of Ad4 infection can be achieved with real-time PCR [19]. In addition, application of this method may contribute to better understanding and management of ARD in military recruit populations.

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