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Rapid Detection of Adenovirus in Throat Swab Specimens by PCR during Respiratory Disease Outbreaks among Military Recruits

Marcela Echavarria,[1] Jose L. Sanchez,[2] Shellie A. Kolavic-Gray,[2] Christina S. Polyak,[2] Felicia Mitchell-Raymundo,[1] Bruce L. Innis,[1] David Vaughn,[1] Richard Reynolds,[3] and Leonard N. Binn[1]*

Department of Virus Diseases, Division of Communicable Diseases and Immunology,[1] and Division of Preventive Medicine,[3] Walter Reed Army Institute of Research, Silver Spring, and U.S. Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, Aberdeen,[2] Maryland

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We evaluated the performance of a generic PCR test to detect adenoviruses (AdV) in throat swab specimens collected from asymptomatic and ill military recruits with acute respiratory disease. Samples (n = 210) were collected at entry to basic training and at the time of large outbreaks of AdV-associated acute respiratory disease among military recruits at Fort Jackson, South Carolina, from 1997 to 1998. Compared to cell culture, a sensitivity of 99% and a specificity of 98% were noted for the PCR method to detect AdV in throat swabs. Similar results were obtained with or without DNA extraction, suggesting the absence of significant inhibitors for the PCR method in throat swab samples. No AdV was detected by culture or PCR in throat swabs from healthy recruits, suggesting the absence of latency or asymptomatic shedding. Throat swab specimens proved to be adequate, noninvasive samples to rapidly diagnose respiratory disease in young adults. This generic direct PCR proved to be a useful test for the rapid diagnosis of AdV-associated respiratory disease, detecting all serotypes tested to date and furnishing results within 6 h of specimen arrival. The use of this direct, rapid, sensitive, and specific assay would assist health care providers and public health practitioners in the early diagnosis, management, and control of AdV-associated respiratory disease.

Adenoviruses (AdV) were first associated with respiratory outbreaks among military recruits undergoing basic training in 1953 (8). During the 1950s through the 1960s, AdV-associated acute respiratory disease (ARD) constituted one of the most important causes of medical morbidity among military recruits in the United States (14). The serotypes most commonly associated with respiratory disease were types 4 and 7, with types 3, 14, and 21 also being detected in these populations. Since 1980, the routine use of U.S. Food and Drug Administration-approved live oral vaccines for serotypes 4 and 7 among military recruits resulted in a significant decrease in ARD morbidity (5). However, in 1996, a discontinuation of production of these two vaccines by its sole manufacturer and subsequent vaccine shortages resulted in a resurgence of AdV-associated respiratory disease epidemics at several military recruit-training centers (6, 7).

AdV infections have a variety of other clinical manifestations, such as pneumonia, cystitis, conjunctivitis, diarrhea, hepatitis, myocarditis, and encephalitis (4). Disease may vary from mild and self-limited to severe, disseminated, and fatal. Despite its relatively common occurrence in infants and young children, fatal pneumonia in healthy adults appears to be rare. Nevertheless, a few fatal cases have been reported among military trainees (12).

To increase sensitivity and provide rapid diagnosis of AdV infections, molecular methods have been developed. A PCR method for rapid detection of AdV in urine and blood has been recently reported for immunocompromised patients at high risk of AdV-associated infection (3). This same generic PCR was found also to be highly sensitive in detecting AdV in environmental (air filter) samples during an outbreak of AdV-associated ARD among military recruits at Fort Jackson, South Carolina (2).

In this study, we evaluated the generic PCR for direct detection of AdV causing respiratory disease in military recruits. We compared PCR to viral culture results, assessed the inhibitory effect of throat swab samples in PCR, and correlated clinical findings with laboratory data. In addition, we searched for AdV carriage in throat specimens by testing samples from asymptomatic recruits entering basic combat training (BCT).

MATERIALS AND METHODS

Populations. Subjects included healthy and hospitalized recruits with febrile ARD who were undergoing 8 weeks of BCT at Fort Jackson, South Carolina. Specimens were obtained during a respiratory outbreak in November 1997 (13) and from a prospective study conducted in October and November 1998 (1). During the 1997 outbreak, 32 throat swab samples were obtained from hospitalized recruits with ARD; in 1998, 94 similar specimens were collected. These specimens were tested for AdV by culture and PCR methods.

In addition, during the 1998 study, throat swab samples were obtained at entry to BCT from 42 asymptomatic recruits and from an equal number of recruits who self-reported respiratory symptoms.

Case definition. ARD was defined as a febrile illness (oral temperature of 100.5°F or higher) and the presence of one or more of the following respiratory symptoms or signs: sore throat, cough, rhinorrhea, nasal congestion, sinus tenderness, rales, rhonchi, or wheezing (13). All recruits meeting this definition of febrile ARD were hospitalized in accordance with guidelines for ARD control in the military (9).

Throat swab samples. Sterile cotton-tipped wooden sterile applicator sticks (Puritan Pur-Wraps; MDCI Ltd., East Grinstead, United Kingdom) were used to

* Corresponding author. Mailing address: Department of Virus Diseases, Walter Reed Army Institute of Research, 503 Robert Grant Ave., Silver Spring, MD 20910. Phone: (301) 319-9045. Fax: (301) 319-9661. E-mail: leonard.binn@na.amedd.army.mil.

TABLE 1. AdV serotypes tested with this generic PCR method

Subgroup	AdV serotype(s)
A	12, 31
B	3, 7, 11, 16, 21, 34, 35
C	1, 2, 5, 6
D	8, 9, 19, 30, 37, 48, 49
E	4
F	40, 41

collect throat swab samples. The swabs were placed into a transport vial containing 5 ml of media (Eagle's minimum essential medium with Earle's balanced salt solution, 10% fetal bovine serum, nonessential amino acids, and 200 U of penicillin/ml, 200 μg of streptomycin/ml, and 0.25 μg of amphotericin B/ml). Each sample vial was vortexed, and the specimen was transferred to cryovials and immediately frozen at −70°C.

Samples were transported on dry ice and processed at the Department of Virus Diseases at the Walter Reed Army Institute of Research.

AdV Culture. AdV isolation and identification tests were done in A549 cells. Following detection of AdV-like cytopathic effect, the cultures were frozen and the harvested virus was identified in a tube neutralization test with 16 to 20 units of reference AdV antiserum from the Centers for Disease Control and Prevention or the National Institutes of Health (13). The median time to recover virus in the throat specimens was 6 days. Culture was considered the "gold standard" to determine sensitivity and specificity of the PCR.

AdV PCR. PCR was performed as previously described (3). Detection of the amplified products was by agarose gel electrophoresis, involving staining with ethidium bromide, and was recorded with a digital microscopy documentation system (Kodak Digital Science DC 120; Kodak, Rochester, N.Y.). To evaluate the possible presence of inhibitors in throat swab samples collected and stored in viral transport media, PCR was performed before DNA extraction and after DNA extraction by using the QIAamp blood kit (Qiagen Inc., Valencia, Calif.). From either the eluted DNA or the unextracted sample (specimen in viral transport media), 10 μl of template was used for the PCR. Each test included negative and positive extraction and PCR controls. Each test included a negative control (water) and positive controls (dilutions of stock AdV 4). Assays were accepted only when all controls gave the expected results. Investigators were blinded to specimen source and culture results. To assess the analytical sensitivity of the test, serial dilutions of the stocks AdV 4 and AdV 7 were tested by PCR. The highest dilution positive by PCR was considered the limit of detection.

AdV reference stocks and clinical strains. To demonstrate the ability of the generic primers to detect AdV from all six subgroups, different serotypes were tested. AdV serotypes previously tested included 1, 2, 3, 5, 4, 7, 8, 11, 12, 16, 19, 21, 30, 34, 35, 37, 48, and 49. In addition, reference AdV serotypes 6, 9, 31, 40, and 41 from the American Type Culture Collection and respiratory isolates of AdV types 4 and 7 from clinical samples, as well as other field isolates and vaccine strains, were tested. These included AdV type 4 Wyeth vaccine, RI 67, Z-G strain and isolate V10822, AdV type 7 Wyeth vaccine, Gomen, AdV type 7a strain S1058, and AdV 7h isolates from a respiratory outbreak. Serial dilutions of quantified stocks of AdV types 4 and 7 vaccine strains (Wyeth Laboratories) were tested to determine analytical sensitivity.

RESULTS

The analytical sensitivity of the PCR was 1.3 and 1.6 PFU for AdV type 4 and AdV type 7 vaccine strains, respectively, on repeated tests. All 23 AdV strains tested from subgroups A through F were amplified with this PCR method (Table 1).

Throat samples from 210 military recruits were tested for AdV by culture and PCR. Of 126 samples from hospitalized recruits, 99 (79%) were AdV culture positive, 100 (79%) were AdV-PCR positive when extracted samples were tested (Table 2) and 98 (78%) were AdV-PCR positive when nonextracted samples were tested. Sensitivity and specificity of the PCR were 99% and 98%, respectively. The correlation between extracted and nonextracted samples was 98%. In general, the intensity of the bands from both the extracted and unextracted amplified PCR products was very strong, although in a few cases, the intensity of the bands was higher on extracted samples.

All samples collected at entry from nonhospitalized self-reported ill recruits and asymptomatic controls were AdV culture negative (Table 2). Of these 84 samples, one was AdV weak positive by PCR.

The microbiological sensitivity of the PCR test was 99 to 100%, regardless of the serotype involved. Of 76 AdV culture-

TABLE 2. AdV detection by culture and PCR in throat swab specimens from military recruits at entry to basic training and during respiratory outbreaks

Recruit group	Yr	Symptom group	PCR result[a]	Culture result		Total	PCR sensitivity (%)[a]	PCR specificity (%)[a]
				Positive	Negative			
Hospitalized	1997	Febrile ARD	Positive	23	0	23		
			Negative	0	9	9		
			Total	23	9	32	100	100
	1998	Febrile ARD	Positive	75	1	76		
			Negative	1	17	18		
			Total	76	18	94	99	94
Tested at entry	1998	Self-reported febrile ARD	Positive	0	1	1		
			Negative	0	41	41		
			Total	0	42	42	—[b]	98
	1998	Asymptomatic controls	Positive	0	0	0		
			Negative	0	42	42		
			Total	0	42	42	—	100
Grand total			Positive	98	2	100		
			Negative	1	109	110		
Total				99	111	210	99	98

[a] PCR results on extracted specimens.
[b] —, not applicable.

positive samples obtained from hospitalized recruits in 1998, 67 (88%) were type 4, 7 (9%) were type 3, and 2 (3%) were type 21. In 1997, the seven adenovirus isolates identified were type 4. Overall, PCR test sensitivity was 100% for type 3, 99% for type 4, and 100% for type 21. Furthermore, this PCR was able to detect AdV in a recruit with dual infection in which AdV growth was inhibited by the presence of herpes simplex virus. The subsequent destruction of herpes simplex virus with chloroform enabled the detection of AdV by culture. In addition, this PCR test was found to be negative in five cases where either poliovirus or picorna-like viruses were recovered in culture from throat swab samples, further demonstrating the specificity of the test (2).

DISCUSSION

Prior to the use of AdV types 4 and 7 vaccines, infections due to these two viruses produced large epidemics of febrile ARD in military recruits during BCT. Following the recent loss of these vaccines, epidemic ARD due to these AdV has recurred in recruits. The need for rapid diagnosis of large numbers of affected recruits led to the evaluation of a generic AdV-PCR (13). This generic PCR detected all AdV strains tested from the six subgroups, including field and vaccine strains of types 4 and 7. The analytical sensitivity of this generic PCR was less than 2 PFU for types 4 and 7, which represent the major viruses responsible for ARD in recruits.

The generic AdV-PCR was evaluated by testing throat swab specimens from 126 hospitalized recruits with ARD. In comparison to cell culture isolation tests, PCR testing of extracted throat swab specimens had 99% sensitivity and 98% specificity. Since nearly identical results were obtained with unextracted specimens, sample extraction can be omitted when using this viral transport medium, enabling considerable labor and cost savings. All three AdV types responsible for these outbreaks (types 4, 3, and 21) that were recovered in cell culture were detected by this PCR. In addition, AdV was detected by PCR in a mixed infection with herpes simplex virus, showing the specificity of the test. The rapid growth of the herpesvirus obscured AdV detection in cell culture until the herpesvirus was inactivated by chloroform.

This generic PCR method applied with throat swab samples was more sensitive and more specific than other PCR methods described for the diagnosis of respiratory AdV infections (sensitivity, 99% versus 76% [10]; specificity, 98% versus 85% [11). The primers used in this PCR amplified a highly conserved region of the hexon gene, allowing amplification of all tested strains, including prototype and clinical isolates. AdV type 4, the major cause of ARD in recruits, was sequenced in the region amplified by this PCR; no mismatches were observed in the region where the primers anneal. However, BLAST search of AdV type 3 and 7 sequences in GenBank revealed the presence of two mismatches in the primer regions, but not at the terminal end. These mismatches appeared to have no effect on the test's diagnostic sensitivity, since all type 3 and type 21 isolates were detected. Technically, the direct PCR described here is simpler, faster, cheaper, and less prone to contamination than the nested PCR procedure, thereby making it more desirable for clinical laboratory diagnosis.

This report is the first describing the performance of a generic direct AdV-PCR testing throat swab specimens from healthy subjects and acutely ill ARD patients. The method proved to be highly sensitive, specific, and rapid. The use of throat swab specimens was sensitive and noninvasive, and specimens were easily obtainable compared to nasopharyngeal specimens that could be tested directly without extraction. Future use of this test in outbreaks of ARD in military recruits will enable physicians to rapidly diagnose AdV illness and thereby contribute to the management and institution of necessary control measures to prevent AdV transmission.

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