Evaluation of Real-Time PCR Assays for Detection of Multiple Adenovirus Serotypes Using the 3M Integrated Cycler Microfluidic System

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Abstract/Introduction

Introduction: There are more than 50 different human adenovirus serotypes that are divided into 6 subgenera, and these serotypes are associated with infections of different systems. Although the hexon gene is relatively conserved among adenovirus serotypes, there is still some minor sequence variation among serotypes. Two different Scorpion primer pairs, one targeting the 5' end and the other targeting the 3' end of the adenovirus hexon gene, were evaluated. Cross-reactivity and analytical detection of the different subgenera was evaluated with individual primer pairs in a rapid real-time PCR system.

Materials and Methods: Samples were generated by spiking individual strains of adenoviruses or potential cross-reacting organisms into viral transport media. These samples were extracted using a Roche MagNA Pure LC instrument and tested with both primer pairs on a rapid real-time PCR system.

Results: No cross-reactivity was observed with other respiratory viruses, relevant organisms and normal flora. Each primer pair efficiently amplified the selected adenovirus serotypes except the 3' hexon primer pair did not amplify serotype 31.

Conclusions: Two primer pairs evaluated in this study were capable of detecting the different subgenera of adenoviruses. Lack of sequence conservation among different adenovirus serotypes could be overcome by using two primer pairs that target different regions of the adenovirus hexon gene.

Methods

Virus strains. The following adenovirus serotypes were used in the study: serotype 1 (Zeptometrix), serotype 5 (Advanced Biotechnologies, Inc.), serotypes 2, 4, 7, 8, 12, 14, 31, 34, 40, 41 (ATCC).

Nucleic acid preparation. For all specimens, 200 µL of each sample was extracted using the Roche MagNA Pure LC automated system with a Total Nucleic Acid Isolation Kit (Roche Diagnostics, Indianapolis, IN), and eluted in 50 µL of elution buffer. A DNA internal control was added to each specimen prior to extraction to monitor the extraction process and PCR inhibition.

Primers. Two Scorpion primers sets were designed to target conserved regions in either the 5' or the 3' region of the adenovirus hexon gene.

Methods (Cont).

Real-time PCR amplification and detection. PCR was carried out with 5 µL of extracted DNA in a total reaction volume of 10 µL. RT-PCR was performed by using a rapid real-time PCR system. Fluorescent signal for target-specific PCR products was detected at 60°C.

Cross-reactivity. Genomic DNA or RNA from a variety of viral pathogens were tested to evaluate cross-reactivity with the adenovirus primer pairs. For each sample, organisms were spiked into a 200 µL aliquot of viral transport media, and extracted nucleic acids were tested with each of the primer pairs.

Detection of Serotypes. A variety of adenovirus serotypes were diluted in transport media, and nucleic acids were extracted. Each extracted sample was tested in duplicate for each serotype, and Ct values were averaged.

Results

Cross-reactivity: Neither Adenovirus primer pair cross-reacted with the pathogens tested (Table 1), and each was specific for adenoviruses. In addition to the laboratory based cross-reactivity studies, database searches (Altschul, S. F., *et al*. Basic Local Alignment Search Tool. *Journal of Molecular Biology* **215**, 403–410, 1990) indicate that the targeted regions do not have significant homology with sequences from other pathogens.

Table 1. Organisms tested for Cross-Reactivity with the Adenovirus 3' and 5' hexon Primer Pairs.

Anaplasma phagocytophilium	Hepatitis D Virus	*Mycoplasma hominis*
Babesia microti	Herpes Virus -6	*Mycoplasma pneumoniae*
Bartonella henselae	Herpes Virus -7	*Neisseria meningitides*
Bartonella quintana	Herpes Virus -8	Parainfluenza type-1
Bordetella parapertussis	HIV-1	Parainfluenza type-2
Bordetella pertussis	HIV-2	Parainfluenza type-3
Borrelia burgdorferi	HSV-1	Parvovirus B19
Chlamydophila pneumoniae	HSV-2	*Pseudomonas aeruginosa*
Coronavirus 229E	HTLV-1	RSV A
Coronavirus OC43	Human metapneumovirus	RSV B
Corynebacterium urealyticum	Influenza A	*Serratia marcesans*
Cytomegalovirus	Influenza B	*Staphylococcus epidermidis*
Echovirus 7	JC Virus	*Streptococcus mutans*
Ehrlichia chaffeensis	*Klebsiella pneumoniae*	*Streptococcus pneumoniae*
Epstein Barr Virus	*Legionella pneumophila*	*Streptococcus salivarius*
Haemophilus influenzae	MRSA	*Toxoplasma gondii*
Hepatitis B Virus	MSSA	Varicella Zoster Virus
Hepatitis C Virus	*Mycobacterium tuberculosis*	

Results (Cont.)

Serotype Coverage: Each primer pair detected at least 12 different serotypes of Adenovirus in the 6 subgenera (Table 2). However, Ct values for each strain varied depending on which primer pair was used, Furthermore, the 3' Hexon primer pair did not detect serotype 31, likely due to sequence mismatches in the primer region.

Table 2. Analytical detection of different serotypes.

Adenovirus		Primer Pair Ct Values		
Subgenera	Serotype	5' Hexon	3' Hexon	Ct Variance
Species A	12	27.4	31.4	4
	31	28.9	Undetermined	N/A
Species B	3	31.5	30.2	-1.3
	7	32.1	31.1	-1
	14	30.7	33.5	2.8
	34	28.3	30.8	2.5
Species C	1	30.5	27.9	-2.6
	2	33.5	34	0.5
	5	33.3	31	-2.3
Species D	8	30.4	30.2	-0.2
Species E	4	32.3	33.1	0.8
Species F	40	24.3	24.3	0
	41	31.2	31.1	-0.1

Conclusions

- Two primer pairs evaluated in this study were capable of detecting the different subgenera of adenoviruses; Ct values varied based on the primer pair used.
- No cross-reactivity was detected for each primer pair for 27 viruses, 24 bacteria and 2 protozoa.

Acknowledgments

The authors would like to thank Jules Chen and Emberlee Eleazar for their assistance and for helpful comments and suggestions.

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