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ORIGINAL ARTICLE

One-Step Reverse Transcription-Polymerase Chain Reaction for Ebola and Marburg Viruses

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Abstract

Objectives: Ebola and Marburg viruses (EBOVs and MARVs, respectively) are causative agents of severe hemorrhagic fever with high mortality rates in humans and nonhuman primates. In 2014, there was a major Ebola outbreak in various countries in West Africa, including Guinea, Liberia, Republic of Sierra Leone, and Nigeria. EBOV and MARV are clinically difficult to diagnose and distinguish from other African epidemic diseases. Therefore, in this study, we aimed to develop a method for rapid identification of the virus to prevent the spread of infection.

Methods: We established a conventional one-step reverse transcription-polymerase chain reaction (RT-PCR) assay for these pathogens based on the Superscript Reverse Transcriptase-Platinum *Taq* polymerase enzyme mixture. All assays were thoroughly optimized using *in vitro*-transcribed RNA.

Results: We designed seven primer sets of nucleocapsid protein (NP) genes based on sequences from seven filoviruses, including five EBOVs and two MARVs. To evaluate the sensitivity of the RT-PCR assay for each filovirus, 10-fold serial dilutions of synthetic viral RNA transcripts of EBOV or MARV NP genes were used to assess detection limits of viral RNA copies. The potential for these primers to cross react with other filoviruses was also examined. The results showed that the primers were specific for individual genotype detection in the examined filoviruses.

Conclusion: The assay established in this study may facilitate rapid, reliable laboratory diagnosis in suspected cases of Ebola and Marburg hemorrhagic fevers.

1. Introduction

Filoviruses are RNA viruses that belong to the family Filoviridae, which includes zoonotic pathogens of Ebola viruses (EBOVs), Marburg viruses (MARVs), and Cuevaviruses. EBOVs and MARVs cause Ebola and Marburg hemorrhagic fevers, respectively [1]. These viruses cause severe and often fatal hemorrhagic fever, with case fatality rates ranging from 25% to 90% depending on the strain or species.

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Currently, EBOVs are subdivided into five species with different pathogenicities [2]. Zaire EBOV (ZEBOV), the most lethal species (case fatality rate of up to 90%), has caused numerous human outbreaks between 1976 and 2008 in the Democratic Republic of the Congo, Republic of the Congo, and Gabon [3,4]. Sudan EBOV (SEBOV; case fatality rate of approx. 50%) has caused three documented outbreaks in Sudan and one in Uganda [5,6]. Taï Forest EBOV (TEBOV; previously known as Côte d'Ivoire Ebola virus) has been linked to a single, nonfatal human case [7], and the newly discovered Bundibugyo EBOV (BEBOV) caused an outbreak with a 25% case fatality rate in 2007 in Uganda [8]. Reston EBOV (REBOV), which has caused outbreaks in nonhuman primates and swine in the Philippines, appears to be nonpathogenic in humans [9].

MARV consists of one species with two members, namely, Ravn virus (RMARV) and MARV; RMARV includes four strains [MARV-Popp, MARV-Musoke (MMARV), MARV-Ozolin, and MARV-Angola] [10]. MARV has been shown to be responsible for at least nine outbreaks since 1967, with four occurring in the past decade, including a recent outbreak that began in September 2012 in Uganda [11]. The increased frequency of MARV outbreaks together with the fact that these viruses are potential agents of bioterrorism has increased public health concern regarding filoviruses.

A number of diagnostic methods are available for the detection and identification of filoviruses. These methods include virus isolation, enzyme-linked immunosorbent assays for detection of antigen or antibodies, reverse transcription-polymerase chain reaction (RT-PCR), and electron microscopy, all of which have played major roles in the diagnosis of filovirus infections. In particular, RT-PCR targeting viral nucleic acid is a rapid, sensitive technique to detect filoviruses. There are a number of commercial and in-house PCR assays for detection of filoviruses with different targets.

In this study, we developed a one-step RT-PCR method using primers for amplifying a specific RNA sequence by expressing the nucleocapsid protein (NP) of EBOV or MARV. Using this method, the presence of EBOV or MARV genes in the samples may be identified more accurately and detected more rapidly. Moreover, through application of specific primers, this method could be used for specific detection of seven types of filoviruses, including the five known EBOVs and two known MARVs.

2. Materials and methods

2.1. Primers design

Nucleotide sequences of the N-protein-encoding segments of seven known EBOVs and MARVs were aligned using the CLUSTAL W multiple alignment algorithm (MegAlign program, Lasergene sequence analysis software; DNASTAR Inc., Madison, WI, USA) to identify conserved regions. Primers for each segment were designed using Primer Express software (Version 3.0; PE Applied Biosystems, Foster City, CA, USA). The specificity of each primer was checked using the Basic Local Alignment Search Tool search against the National Center for Biotechnology Information database and then appraised using a primer selection program in Lasergene software. The sequences and details of primers are listed in Table 1.

2.2. Synthesis of RNA transcripts

Seven templates from the MMARV NP gene (2,088 nt), the SEBOV NP gene (2,217 nt), the TEBOV NP gene (2,220 nt), the BEBOV NP gene (2,220 nt), the

Table 1. Primers for reverse transcription-polymerase chain reaction used in the study.

Virus	Primer	Sequence (5′→3′)	Position	Size (bp)
BEBOV	Forward	GCAGAAATATGCTGAATCTCGTGAAC	1062	418
	Reverse	ATCATCCTCGTCCTCAAGGTCAAAA	1479	
REBOV	Forward	CCAACAATATGCTGAGTCCAGAGAA	1062	419
	Reverse	CATCCTCATGATCGTCAAGATCG	1480	
SEBOV	Forward	ACACGTGAGTTGGACAACCTT	1078	402
	Reverse	GTCATCGTCGTCGTCCAAATTGAA	1479	
TEBOV	Forward	AATCTCGCGAGCTTGACCAT	1076	404
	Reverse	CTCGTCACCATCTTCAAGGTCAAA	1479	
ZEBOV	Forward	CGAACTTGACCATCTTGGACTTG	1083	399
	Reverse	TCCTCGTCGTCCTCGTCTAGAT	1481	
MMARV	Forward	AGGCGACATGAACATCAGGAAATT	1012	398
	Reverse	TCGTCCTCATTCAGCAGTGCAAAT	1409	
RMARV	Forward	GCGACATGAACACCAGGAAATTC	1014	412
	Reverse	ATTTTCAAGAGTATCCTCGTCTTCG	1425	

BEBOV = Bundibugyo EBOV; bp = base pair; MARV = Marburg virus; MMARV = MARV-Musoke; REBOV = Reston EBOV; RMARV = Ravn virus; SEBOV = Sudan EBOV; TEBOV = Taï Forest EBOV; ZEBOV = Zaire EBOV.

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