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Rapid detection of HIV-1 by reverse-transcription, loop-mediated isothermal amplification (RT-LAMP)☆

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ABSTRACT

A rapid, cost-effective diagnostic or confirmatory test for the detection of early HIV-1 infection is highly desired, especially for use in resource-poor or point-of-care settings. The reverse-transcription loop-mediated isothermal amplification (RT-LAMP) technology has been evaluated for the detection of HIV-1 DNA and RNA, using six RT-LAMP primers designed against highly conserved sequences located within the protease and p24 gene regions. Amplification from lab-adapted HIV-1 DNA and RNA was detected as early as 30 min, with maximum sensitivity of 10 and 100 copies per reaction, respectively, reached at 60 min. Comparable sensitivity was observed with extracted nucleic acid from plasma and blood samples of HIV-1-infected individuals. Furthermore, the RT-LAMP procedure was modified for the direct detection of HIV-1 nucleic acid in plasma and blood samples, eliminating the need for an additional nucleic acid extraction step and reducing the overall procedure time to approximately 90 min.

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1. Introduction

Nearly 25 years since the discovery of the etiologic agent of AIDS, the HIV pandemic continues to be a major public health concern. As there is currently no available vaccine for HIV-1, much emphasis has been placed on the development of diagnostic tests to assist in the evaluation of various intervention strategies. Additionally, diagnosis of acute HIV-1 infection may have important implications for reducing the dissemination of the virus. During acute HIV infection, infected individuals are at a higher risk for transmitting the virus due to peak viral levels in blood and genital secretions prior to the development of an HIV-specific immune response (Pilcher et al., 2001). Though identification of early infection and intervention with antiretroviral therapy or risk-reduction counseling may reduce HIV transmission, only a very small percentage of acutely infected individuals are diagnosed within the first month of infection (Patel et al., 2006; Pilcher et al., 2004).

The importance of diagnostic HIV tests for controlling the HIV epidemic is evidenced by the numerous tests currently licensed by the U.S. Food and Drug Administration, each having its own strengths and weaknesses (Ketema et al., 2005). Despite the evolving array of HIV diagnostic tests available, HIV testing algorithms have not been updated since the late 1980s. Based on the previous CDC/ASTPHLD (Association of State and Territorial Public Health Laboratory Directors) guidelines, HIV testing in large populations of patients involves frequently some combination of antibody-based enzyme immunoassay (EIA) test, followed by confirmation by Western Blot or indirect immunofluorescence assay (IFA) (Anon., 1989). The use of rapid HIV tests is highly attractive for screening of patient samples, especially in developing countries where resources are limited, because they are quick, easy to perform, and do not require special equipment. Rapid tests for the identification of HIV antibody, however, will remain negative during the four to five week window post-infection and pre-seroconversion, necessitating diagnosis based on p24 antigen or HIV-1 nucleic acid (Fiebig et al., 2003; Fiscus et al., 2007). HIV p24 antigen-based tests are attractive for diagnosis of acute infection pre-seroconversion, given that p24 antigen can be detected as early as two weeks post-infection (Weber, 2006). Due to the short window of peak viremia, antigen-based tests are relatively insensitive and are used rarely as primary screening tests for HIV (Iweala, 2004). For this reason, the EIA remains the "gold standard" for rapid, large-scale screening of clinical samples. While the EIA is highly sensitive and relatively inexpensive, nucleic acid-based detection methods, such as PCR and RT-PCR, yield a positive result earlier in infection (Daar et al., 2001). With most HIV RNA detection assays, virus in plasma can be detected about seven days prior to p24 antigen and about 12

☆ The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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days prior to antibody detection tests (Fiebig et al., 2003). Current PCR techniques, however, are not feasible screening approaches for developing countries or point-of-care testing due to personnel training requirements and the timely and expensive procedure, requiring sample processing, nucleic acid extraction, and multiple amplification steps. Furthermore, as compared to the EIA, HIV RNA assays are less specific, yielding as high as 1% false-positive rates (Hecht et al., 2002; Pilcher et al., 2004).

A rapid nucleic acid detection method, termed loop-mediated isothermal amplification (LAMP) has been developed by Notomi et al. (2000). LAMP is a one step amplification reaction that amplifies a target DNA sequence with high sensitivity and specificity under isothermal conditions and exhibits sensitivity similar to traditional PCR. LAMP utilizes a DNA polymerase with strand displacement activity, along with two inner primers (FIP and BIP) and two outer primers (F3 and B3), specially designed for six specific regions within the target sequence. Furthermore, an accelerated LAMP procedure has been developed that employs two additional primers (LoopF and LoopB) for enhanced specificity and reaction efficiency (Nagamine et al., 2002). The LAMP technology has also been adapted for the detection of RNA viruses using reverse transcription loop-mediated isothermal amplification (RT-LAMP), simply through the addition of a heat- stable reverse transcriptase (Hong et al., 2004; Kurosaki et al., 2007; Soliman and El-Matbouli, 2006; Yoshida et al., 2007). RT-LAMP is a cost-effective alternative to PCR for detection of viral RNA in resource-limited settings, as the amplification reaction can be carried out in a heat block, obviating the need for a thermal cycler. Additionally, identification of amplified LAMP products can be observed visually due to turbidity caused by magnesium pyrophosphate, a byproduct of the amplification reaction. Direct visual identification can be further enhanced by addition of an intercalating, fluorescent dye, such as PicoGreen, that can be observed under a UV lamp. The applicability of LAMP for resource-limited settings has been demonstrated by Boehme et al. (2007) for diagnosis of tuberculosis.

For the first time, the LAMP technology has been applied to the development of a rapid, nucleic acid-based test for the detection of HIV-1 nucleic acid present in plasma and whole blood of infected individuals. A quick and simple method has been developed for testing small volumes of HIV-1-infected plasma or blood without the need for nucleic acid extraction. Primers directed against highly conserved regions within the protease and p24 genes were designed and used successfully to amplify HIV-1 DNA and RNA using RT-LAMP. Although the addition of reverse transcriptase is not required for amplification of DNA, RT-LAMP was used to amplify all sample types in this study, since both DNA and RNA can be amplified by RT-LAMP.

2. Materials and methods

2.1. Plasma and blood samples

HIV-1-seropositive plasma samples with known viral loads were obtained from ZeptoMetrix Corp., Buffalo, NY (Seroconversion Panel Donor Nos. 62357, 60772, 65389, 68106, 65522). HIV-infected whole blood samples were specimens left over from the diagnostic laboratory and were unlinked from personal identifiers before use (CDC IRB protocol #1896).

2.2. RT-LAMP primer design

The four primers required for the LAMP reaction are designed against six arbitrary regions within the target sequence, termed F3, F2, F1, B3, B2, and B1. The forward outer (F3) and backward outer (B3) primers contain the F3 and B3 sequences, respectively. The forward inner (FIP) and backward inner (BIP) primers are composed of the F2/B2 sequence and the complementary sequence of F1/B1 (F1c/B1c). Additionally, loop primers (LoopF and LoopB) are included to accelerate the LAMP reaction. The loop primers recognize the region between F2/B2 and F1/B1. A schematic representation of the primer design for LAMP is illustrated in Fig. 1. The amplification cycle of accelerated LAMP has been diagramed in detail by Nagamine et al. (2002).

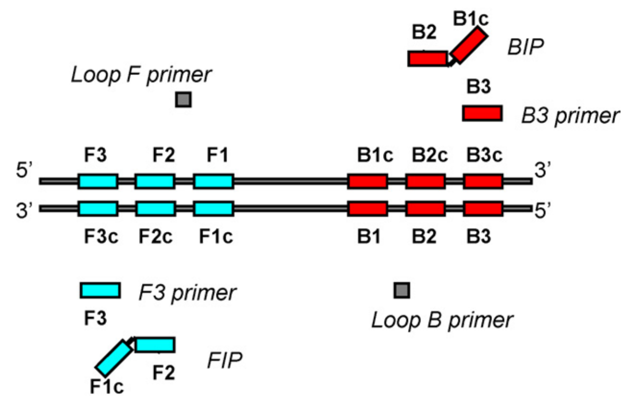

Fig. 1. Schematic representation of RT-LAMP primer design. Two inner primers (FIP and BIP) and two outer primers (F3 and B3) are designed against six arbitrary regions within the target DNA sequence (F3, F2, F1, B3, B2, and B1). Loop primers (LoopF and LoopB) are composed of the complementary sequence of the region between F2 and F1 or B2 and B1, respectively.

HIV-1-specific F3, B3, FIP, BIP, LoopF and LoopB primers were designed using the PrimerExplorer V3 software available on the Eiken Chemical Co. Ltd. website (http://primerexplorer.jp/e/). A spacer of four thymidines was inserted between F2/B2 and F1c/B1c sequences of the FIP and BIP primers, as described (Notomi et al., 2000). Two sets of RT-LAMP primers were designed, each recognizing a target sequence located within either the HIV-1 protease or p24 gene. The HIV-1 BaL sequence (GenBank accession number AY713409), chosen as a representative clade B strain, was used as a reference for generating both sets of primers (Brown et al., 2005). All RT-LAMP primers were obtained from Sigma–Genosys (St. Louis, MO). The sequences of the protease and p24-specific primer sets are shown in Table 1.

Table 1
RT-LAMP primers for the detection of HIV-1

Target gene	Primer name	Sequence (5′ to 3′)
p24	F3	ATTATCAGAAGGAGCCACC
	B3	CATCCTATTTGTTCCTGAAGG
	FIP	CAGCTTCCTCATTGATGGTTTCTTT TTAACACCATGCTAAACACAGT
	BIP	TGTTGCACCAGGCCAGATAATTTT GTACTGGTAGTTCCTGCTATG
	LoopF	TTTAACATTTGCATGGCTGCTTGAT
	Loop B	GAGATCCAAGGGGAAGTGA
Protease	F3	AAAGATAGGGGGGCAACT
	B3	GTTGACAGGTGTAGGTCCTA
	FIP	GGTTTCCATCTTCCTGGCAAATTTT TTCTCTATTAGATACAGGAGCAGA
	BIP	TGATAGGGGGAATTGGAGGTTTTT TTCCTATAGCTTTATGTCCACAGA
	Loop F	TATTTCTTCTAATACTGTATCA
	LoopB	TATCAAAGTAAGACAGTA

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