

IN-HOUSE RT-PCR ASSAY FOR DETECTION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 INFECTION

Andi Yasmon, Ni Nengah Dwi Fatmawati, Fera Ibrahim, Ketut Tuti Parwati, Budiman Bela

Department of Microbiology, Medical Faculty, University of Indonesia, Jakarta 10320, Indonesia

E-mail: andiyasmon@yahoo.com

Abstract

Serologic assays are commonly used for screening (ELISA) and for confirmation (Western blot) of HIV-1 infection; however, both assays have potentially yielded the false-positive or false-negative results. In this study, a diagnostic RT-PCR assay as an alternative test for detection of HIV-1 was developed. Forty-six plasma specimens from highly risky groups, who visited a voluntary counseling and testing for HIV (VCT) in Sanglah Clinic of General Hospital, Denpasar, Bali, were tested by RT-PCR assay with specific primers for *Pol* region of HIV-1 genome. The results of the RT-PCR tests were then compared with those of serologic tests to obtain the sensitivity and specificity of RT-PCR assay. The results of this study showed that the RT-PCR assay could detect 17 (sensitivity: 65.4%) of 26 serologically positive specimens and was unexpectedly able to detect 2 (specificity: 90%) of 20 serologically negative specimens. Thus, the RT-PCR assay developed in this study is potential to be used as an alternative test, even though there are numerous aspects, particularly the sensitivity, that need to be improved in further research.

Keywords: HIV-m, RT-PCR, serologic assay

Introduction

Since Acquired Immunodeficiency Syndrome (AIDS) disease was known, it has been the most important concern to public health around the world.^{1, 2} Based on UNAIDS data, it was globally reported that by the end of 2007 there were 33.2 million HIV-1 infection cases, and every day more than 5.700 people died from AIDS.³ In Indonesia, the HIV epidemic is among the fastest growing in Asia in that HIV infections occur through the use of contaminated injecting equipment, unprotected paid sex and, to a lesser extent, unprotected sexual activities between men.⁴ Bali is one of provinces in Indonesia with high HIV epidemic.⁵

Enzyme-linked immunoassay (ELISA) is commonly used for screening HIV-1 infection, whereas Western blot assay (WB) is only used for confirmation of HIV-1 infection. Both ELISA and WB assays detect the HIV antigens or the HIV specific antibodies. The inferior of such both assays always is concerning false-positive or false-negative results.⁶ The false positive is the result of unspecific binding of the HIV specific antibodies to non-HIV proteins, or vice versa. While the false negative can be caused by: 1) infected individuals fail to produce HIV-1 specific antibodies⁷; 2) in cases of infection with the most divergent HIV-1 strains⁸; 3) at early phase of infection when HIV-1 specific antibody is not produced

yet⁹; and 4) seronegativity or immunological dysfunction caused by the aggressive disease course. WB is especially not often performed because it is more expensive.⁶

Reverse transcription-polymerase chain reaction (RT-PCR) is a known technique to amplify particular RNA directly. This technique is more suitable because it is able to detect HIV RNA in the early phase of infection even when HIV-1 specific antibody of a newly HIV-1 infected person is not produced yet, or in the late phase in which antibody is unable to be detected.⁹ Therefore, in this study a diagnostic RT-PCR was applied to amplify RNA of HIV-1 genome using primer specific to *Pol* region of HIV-1 genome. It was expected that this assay could be used as an alternative test for detection of HIV-1 infection and as part of efforts to reduce transmission of HIV-1 especially in Indonesia.

Methods

Forty-six blood specimens, from high-risk patients (intravenous drug users, female sex workers, widespread sexual risk behaviors, homosexuals, recipients of blood transfusion, and babies with HIV-1 positive mothers), were collected from July to August 2005 at VCT of RSUP Sanglah, Bali. Blood specimens were collected into a vacutainer without EDTA for

serologic assay and into a vacutainer with EDTA for RT-PCR assay. For serologic assay, specimens were tested in VCT of Clinic Sanglah General Hospital-Bali by three different rapid test kits i.e. Determine™ HIV ½ (Abbott), ImmunoComb® HIV 1&2 BiSpot (Orgenics), and Serodia® HIV ½ (Fujirebio Inc.). HIV-1 positive was defined as serum reactive against two or three rapid test kits.

HIV-1 RNA(s) were extracted and purified by QIAamp Viral RNA Mini Kit (Qiagen®) according to the manufacturer's instruction. One-tube RT-PCR reactions were performed by using OneStep RT-PCR Kit (Qiagen®) with the following compositions: 1x OneStep RT-PCR Buffer, 2.5 mM MgCl₂, 1x Q solution, 400 μM each of dGTP, dCTP, dTTP and dATP, 0.6 μM each of forward (5'-CCC TCA GGT CAC TCT TTG G-3' and 5'-CCC TCA AAT CAC YYT TTG G-3') and reverse primers (5'-ATT CCC CCT ATC ATT TTT GG-3' and 5'-ATT CCC CCT ATC ATY YTT GG-3'), OneStep RT-PCR enzyme mix, 4 μL viral RNA, and added nuclease-free water until final volume of 10 μL. Thermal cycler, AB Applied Biosystems Gene Amp PCR System, was used with cycles: reverse transcription for 30 min at 50 °C; initial PCR activation step for 15 min at 95 °C; 40 cycles of denaturing for 30 sec at 94 °C, annealing for 30 sec at 56 °C, and extension for 30 sec at 72 °C; and final extension for 7 min at 72 °C. RT-PCR products were analyzed on 8% polyacrylamide gel and visualized on ultraviolet light.

The results of RT-PCR tests were compared with those of serological tests to obtain the sensitivity and specificity of RT-PCR assay.

Results and Discussion

A positive RT-PCR assay was defined by a 150 bp DNA fragment visualized at the right position on polyacrilamide gel (Figure 1). Optimization of annealing temperature (AT) for PCR reaction was performed by using pNL43 as template. This result showed that the primers could amplify DNA at 57,9 °C, 56,1 °C, 54,8 °C, and 54 °C with optimal amplification at 56,1 °C (data not shown).

In this study, the RT-PCR assay could detect 17 of 26 serologically positive specimens (sensitivity: 65.4%). Of 20 serologically negative specimens, the RT-PCR assay showed 18 negative (specificity: 90.0%) and 2 positive results (false positive: 10.0%) (Table 1). The results disagree with what was reported by Hecht *et al.*¹⁰ in that their RT-PCR assays were able to detect primary HIV infection with 100 percent of sensitivity. The different results could be due to the strategic differences of RT-PCR techniques especially the type of used primer, and stage of HIV-1 infection when blood is collected. In addition, a particular stage of HIV-1 infection also

influences sensitivity of RT-PCR assay. As having been reportedm viremia peak of HIV-1 infection commonly occurs at the beginning stage before serologic conversion or AIDS.¹¹ In this study, we used plasma obtained from the HIV-1 infected patients with clinically variable symptoms; it is thought that the stage of HIV-1 infection could influence sensitivity and specificity of our technique. This thought might explain why RT-PCR technique reported by Hecht *et al.*¹⁰ gave 100 percent of sensitivity, possibly because of only diagnosing the HIV patients with primary infection. Thus, we propose that the stage of HIV-1 infection must be considered when evaluating a diagnostic RT-PCR.

Moreover, RT-PCR assay was unexpectedly able to detect 2 of 20 serologically negative specimens (Table 1). The results might depict a real true-positive test particularly in patients without seroconversion yet at the initial infection or having an immunological system which failed to stimulate the antibody production at the AIDS stage.¹² In those conditions, the rapid assay is not able to diagnose the patients. These reasons may explain why the commercially rapid tests e.g., ImmunoComb® HIV 1&2 BiSpot and Serodia® HIV ½ (Fujirebio Inc.) could yield false negative tests.^{12,13}

While the sensitivity and specificity value obtained by comparing RT-PCR assay with serological assay, the validity of this developing RT-PCR mostly depended on how much the sensitivity and specificity of serologic assay as a confirmation assay was. It was previously reported that commercially rapid tests such as

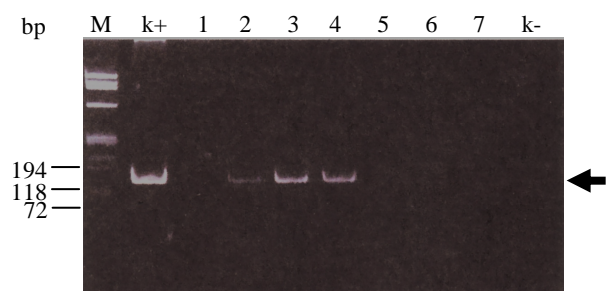


Figure 1. Results of RT-PCR Assays. M: DNA Ladder. Line 1-7: Seven Examples of Tested Samples. Line 2-4: Positive Samples. Line 1,5-7: Negative Samples. k-: Negative Control. k+: Positive Control. bp: Base Pair

Tabel 1. Result of RT-PCR Assay Compared with Serologic Assay

	Serology		Total
	Reactive	Non Reactive	
RT-PCR Positive	17	2	19
RT-PCR Negative	9	18	27
Total	26	20	46

ImmunoComb® HIV 1 & 2 BiSpot, Serodia® HIV ½ (Fujirebio Inc.), and Determine™ HIV ½ (Abbott) were also able to yield false positive tests.¹²⁻¹⁴ This disadvantage might be one of many factors leading the sensitivity of our assay to be low. This assumption has to be confirmed by a technique like real time RT-PCR that is much more sensitive and specific.

Conclusion

Even though the sensitivity (65.4%) of diagnostic RT-PCR assay developed in this study was low, use of molecular method for detection of HIV-1 seems justified in a diagnostic approach. Molecular diagnosis defined in this study has a major advantage: it is simple to carry out with high specificity, even though there are numerous aspects, e.g. type of primer and its target, that need to be optimized in order the sensitivity to be improved.

Acknowledgements

This research has been funded by Riset Unggulan Universitas Indonesia (RUUI). We are grateful to the VCT of Sanglah Clinic of General Hospital, Denpasar-Bali for providing plasma samples.

References

- Brooks GF, Butel JS, Morse SA. *Jawetz, Melnick and Adelberg's medical microbiology*. 23 ed: Boston/Toronto: McGrawHill; 2004.
- Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. *Medical microbiology*. 4 ed: St. Louis/Toronto: Mosby; 2002.
- (WHO). HAUaWHO. *AIDS epidemic update*; December 2007.
- Surveillance UWWGoGHAS. *Guidelines for using HIV testing Technologies in Surveillance*; 2001.
- (WHO) JUNPoHAUaWHO. *ASIA: AIDS epidemic update: Regional Summary*; 2008.
- (UNAIDS) TJUNPoHA. *Knowing your epidemic*. UNAIDS Annual Report:2008.
- Cardoso AR, Goncalves C, Pascoalinho D, Gil C, Ferreira AF, Bartolo I, et al. Seronegative infection and AIDS caused by an A2 subtype HIV-1. *AIDS* 2004; 18: 1071-4.
- Simon F, Mauclore P, Roques P, Loussert-Ajaka I, Muller-Trutwin MC, Saragosti S, et al. Identification of a new human immunodeficiency virus type 1 distinct from group M and group O. *Nat Med* 1998; 4: 1032-7.
- Mylonakis E, Paliou M, Lally M, Flanigan TP, Rich JD. Laboratory testing for infection with the human immunodeficiency virus: established and novel approaches. *Am J Med* 2000; 109: 568-76.
- Hecht FM, Busch MP, Rawal B, Webb M, Rosenberg E, Swanson M, et al. Use of laboratory tests and clinical symptoms for identification of primary HIV infection. *AIDS* 2002; 16: 1119-29.
- Cohen OJ, Fauci AS. Pathogenesis and Medical Aspects of HIV-1 Infection. In: *Fields Virology*. 4 ed: Philadelphia: Lippincot Williams & Wilins 2001.
- Chin BS, Lee SH, Kim GJ, Kee MK, Suh SD, Kim SS. Early identification of seronegative human immunodeficiency virus type 1 infection with severe presentation. *J Clin Microbiol* 2007; 45: 1659-62.
- Aghokeng AF, Ewane L, Awazi B, Nanfack A, Delaporte E, Peeters M, et al. Evaluation of four simple/rapid assays and two fourth-generation ELISAs for the identification of HIV infection on a serum panel representing the HIV-1 group M genetic diversity in Cameroon. *J Acquir Immune Defic Syndr* 2004; 37: 1632-40.
- Lien TX, Tien NT, Chanpong GF, Cuc CT, Yen VT, Soderquist R, et al. Evaluation of rapid diagnostic tests for the detection of human immunodeficiency virus types 1 and 2, hepatitis B surface antigen, and syphilis in Ho Chi Minh City, Vietnam. *Am J Trop Med Hyg* 2000; 62: 301-9.