

LONG TERMINAL REPEATS REGION AS A POTENTIAL TARGET FOR HIV MOLECULAR DETECTION

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Abstract

Indonesian National Committee for human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) has reported the significant increase of HIV infected individual in Indonesia. A sensitive accurate diagnostics are urgently needed to prevent the dissemination of HIV and also to provide a suitable therapy. For this reason, we have developed HIV diagnostic method based on PCR to elucidate this problem. For this research, samples were collected from hospitals and Indonesian Red Cross that consist of samples possessing HIV serological test positive and indeterminate. Ribonucleic Acid (RNA) were isolated from blood plasma. These RNA then were amplified after Reverse transcriptase reaction by using primers which have been designed using *env* (C2V3C3), Long Terminal Repeats (LTR) (U3) and Capsid gag (p24) as target regions. The obtained results shown 26/34 (76.5%) positive in LTR, 10/33 (36.4%) positive in Env and 11/33 (33.3%) positive in p24. These results showed that LTR primers detect HIV more than other primers. It suggests that LTR could be used as detection target as complement of *env* and p24. These results need to be explored further by using sequencing method.

Keywords: *env*, *gag*, *HIV*, *LTR*

Introduction

In Indonesia, human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) cases are increasing significantly in the last recent years. This increase caused majority by the increasing of IDU users in Indonesia. The emerging HIV epidemics in countries of Asia and Eastern Europe will contribute significantly to the future of the HIV pandemic. An aggressive action is required either in prevention as development diagnostic methods, vaccines etc. or in treatment as the developments of antivirals using various substance etc. to prevent pandemic.^{1,2}

Since 1986, a number and variety of commercial assays have been available to screen blood, diagnose infection, and monitor disease progression in individuals infected by human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2). These assays are categorized in four main classes, including tests that detect HIV antibody, detect p24 antigen, detect or quantify viral nucleic acids, and estimate T-lymphocyte numbers (cell phenotyping). The enzyme-linked immunosorbent assay (ELISA) is the most common immunoassay utilized for the detection of HIV antibody and antigen. This technique has evolved from the first-generation viral lysate-based immunoglobulin G (IgG) tests, to the second-generation tests incorporating

recombinant and/or synthetic peptide antigens, to the third-generation tests which detect IgG and IgM (antigen sandwich techniques), and finally to the third-generation-plus assays which also detect HIV-1 group O.³

HIV diagnostics by using RT-PCR (Reverse transcriptase polymerase chain reaction) have been developed to increase the sensitivity of HIV detection.^{4,6} Affordable RT-PCR method is required for HIV prevention and antiretroviral treatment programs. Since many non-B subtypes, circulating recombinant forms and unique recombinant forms circulate in these areas, in-house HIV-1 RNA RT-PCR assays are ideal academic tools to thoroughly evaluate the impact of HIV-1 genetic diversity on the accuracy of HIV-1 RNA quantification, as compared with licensed techniques.⁴

This research aimed to develop HIV diagnostic method based on Circulating HIV strains in Indonesia to obtain more accurate and affordable HIV diagnostic methods.

Methods

In this research, blood samples possessing HIV serological test positive and indeterminate were collected from Indonesia Red Cross (PMI) Blood Transfusion Centre (UTDP) Jakarta. Meanwhile, blood samples with

the same criteria also were collected from patients having HIV clinical manifestation. By using Viral RNA isolation kit, HIV RNA were isolated from blood plasma. Target genes then were amplified by using appropriate primers that were designed prior to this research. These designed primers will be developed for HIV diagnostic with focus on circulating HIV in Indonesia.

Samples were collected from Indonesian Red Cross and hospitals. Samples collected were samples possessing HIV serological test positive and indeterminate. Samples were tested by using western blot for confirmation.

Viral RNA were extracted from blood plasma sample by using a RNA isolation kit. Viral RNA were then used for RT-PCR by using an appropriate primers which have been design previously. Primers were designed based on HIV sequences obtained from HIV compendium, published by NIH (National Insitute of Health) with focus on HIV subtype in Thailand which is supposed closed to HIV strain in Indonesia. Amplification were performed in *env* (C2V3C3), *LTR* (U3)/ and Capsid *gag* (protein p24) regions. PCR product were separated by electrophoresis on a 12% polyacrylamide gel. The gels were then stained with ethidium bromide and visualized under a UV transilluminator.

Results and Discussion

For this research, total samples of 34 were collected that consist of 12 samples from HIV positive patients, 1 sample from HIV indeterminate patient, 11 samples from HIV positive blood donor and 10 samples from HIV indeterminate blood donor. Ribonucleic acid (RNA) form blood plasma were isolated from each sample by using viral RNA extraction (Qiagen). These RNA were used for RT-PCR reaction template by using One-Step RT-PCR (Qiagen). Virion RNA reflects currently replicating virus and thus has more pathogenic significance than the proviral DNA present in PBMC (Pheripheral Blood Mononuclear Cells). Proviral DNA contains high levels of integrated defective viral sequences. However, using RNA as the template has technical difficulty because of RNA unstability.⁷ RT-PCR results in HIV positive patient samples showed 10/12 (83%) positive in LTR, 3/7 (42%) positive in *env* and 3/12 (25%) positive in p24. Sample from patient having HIV serological test indeterminate cannot be detected in all gene target regions. Whereas in HIV positive blood donor samples 11/11 (100%), 7/10 (70%) and 8/10 (80%) positive bands in LTR, *env* and p24, respectively, were detected. On the contrary, HIV indeterminate blood donor samples only can be detected 5/10 (50%) in LTR region. In total samples, 26/34(76.5%), 10/33 (36.4%) and 11/33 (33.3%) can be detected in LTR, *env* and p24, respectively. It means that LTR

detect more samples than other target regions. P24 is commonly used as a target for HIV detection method because it is a conserved region.^{8,9} Eventhough, the use of another target region as LTR may necessary to improve

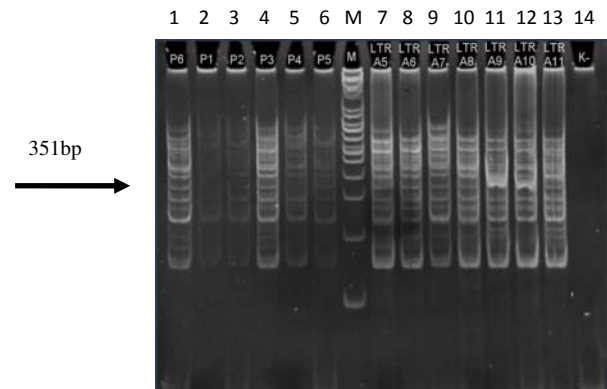


Figure 1. The Amplification of LTR Region (351bp) by Using RT-PCR. PCR Product were Detected by Electrophoresis on 12 % Acrylamide Gel and Visualized by Ethidium Bromide. Column 1: Pos Blood Donor 1, Column 2: Ind. Blood Donor 1, Column 3: Ind Blood Donor 2, Column 4: Ind Blood Donor 3, Column 5: Ind Blood Donor 4, Column 6: Ind Blood Donor 5, Column 7: Pos Patient 5, Column 8: Pos Patient 6, Column 9: Pos Patient 7, Column 10: Pos Patient 8, Column 11: Pos Patient 9, Column 12: Pos Patient 10, Column 13: Pos Patient 11, Column 14: Negatif Control. M: 100bp Marker

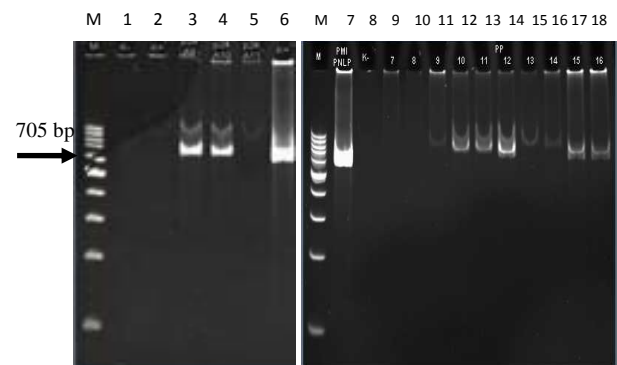


Figure 2. The Amplification of p24 Region (705 bp) by Using RT-PCR. PCR Product were Detected by Electrophoresis on 12 % Acrylamide Gel and Visualized by Ethidium Bromide. Column 1: Negatif Control, Column 2: Positif Control, Column 3: Pos Patient 9, Column 4: Pos Patient 10, Column 5: Pos Patient 11, Column 6: Positif Control, Column 7: Positif Control, Column 8: Negatif Control, Column 9: Pos Blood Donor 2, Column 10: Pos Blood Donor 3, Column 11: Pos Blood Donor 4, Column 12: Pos Blood Donor 5, Column 13: Pos Blood Donor 6, Column 14: Pos Blood Donor 7, Column 15: Pos Blood Donor 8, Column 16: Pos Blood Donor 9, Column 17: Pos Blood Donor 10, Column 18: Pos Blood Donor 11

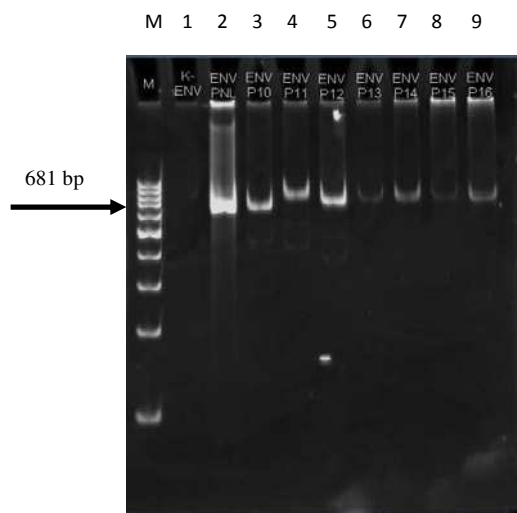


Figure 3. The Amplification of Env Region (681 bp) by Using RT-PCR. PCR Product were Detected by Electrophoresis on 12 % Acrylamide gel and visualized by Ethidium Bromide. Column 1: Negatif Control. Column 2: Positif Control. Column 3: Pos Blood Donor 10, Column 4: Pos Blood Donor 11, Column 5: Pos Blood Donor 12, Column 6: Pos Blood Donor 13, Column 7: Pos Blood Donor 14, Column 8: Pos Blood Donor 15, Column 9: Pos Blood Donor Pos 16

improve the performance of HIV diagnostic. It has been reported that the LTR-based HIV-1 RNA RT-qPCR has been evaluated for HIV diagnostic. The usefulness of this method has been clearly demonstrated for early diagnosis of pediatric HIV-1 infection and monitoring.⁶

LTR region is potential to be developed as target regions of HIV diagnostic as complement of p24 and env. Further experiments should be performed to elucidate the problem of undetectable samples in p24 and env regions. Sequencing method will be necessary to analyze the annealing of each primer and also to analyze the possibility of mutation and escape mutant that may be present in circulating HIV strain in Indonesia.

Conclusion

LTR region is potential to be developed as target region of HIV diagnostic by using RT-PCR as complement of p24 and env target region. Results that showed the small percentage of positive bands in p24 and env target

regions need to be explored further to know the possibility of mutations and escape epitopes. Primers that have been developed in this research require further optimization especially on the annealing site of each primer to improve the accuracy and sensitivity of this method.

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References

1. Grassly NC, Morgan M, et al. Uncertainty in estimates of HIV/AIDS: the estimation and application of plausibility bounds. *Sex Transm Infect* 2004; 80 Suppl 1:i31-38.
2. Ruxrungtham K, Brown T, et al. HIV/AIDS in Asia. *Lancet* 2004; 364(9428):69-82.
3. Saville D, Constantine NT, et al. Fourth-generation enzyme-linked immunosorbent assay for the simultaneous detection of human immunodeficiency virus antigen and antibody. *J Clin Microbiol* 2001; 39(7):2518-24.
4. Bosevska G, Panovski N, et al. RT-PCR detection of HIV in Republic of Macedonia. *Bosn J Basic Med Sci* 2008; 8(4):350-5.
5. Ren A, Louie B, et al. Screening and confirmation of human immunodeficiency virus type 1 infection solely by detection of RNA. *J Med Microbiol* 2008; 57(Pt 10):1228-33.
6. Rouet F, Menan H, et al. In-house HIV-1 RNA real-time RT-PCR assays: principle, available tests and usefulness in developing countries. *Expert Rev Mol Diagn* 2008; 8(5):635-50.
7. Nadai Y, Eyzaguirre LM, et al. Protocol for Nearly Full-Length Sequencing of HIV-1 RNA from Plasma. *PLoS One* 2008; 3(1):e1420.
8. Jeeninga RE, Hoogenkamp M, et al. Functional differences between the long terminal repeat transcriptional promoters of human immunodeficiency virus type 1 subtypes A through G. *J Virol* 2000; 74(8):3740-51.
9. Naghavi MH, Schwartz S, et al. Long terminal repeat promoter/enhancer activity of different subtypes of HIV type 1. *AIDS Res Hum Retroviruses* 1999; 15(14):1293-303.