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Molecular diagnosis of human immuno deficiency virus (HIV) by direct PCR and SEMI nested PCR methods and semi quantification by agarose gel electrophoresis

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ABSTRACT

mPCR is a sensitive assay and could be used as an accurate diagnostic method for detecting various types of microorganisms' genome in low concentration in biological specimens. The demand for sensitive, rapid, safe and easy detection of PCR products has led researchers to a combination of this method with Rapid Card or ELISA. Conserved sequences were selected for design of semi nested primers. Samples were tested by RAPID Card and real- time PCR for detection of specific nucleic acid and viral genome respectively. Viral genome was extracted and reverse transcription was performed with M-Mulv and the cDNA kept at -80º C. The semi nested PCR products were analyzed by running on an agarose gel electrophoresis. Fifteen samples were tested with the Semi Nested PCR method. False positive or negative reactions were not observed. The results from other methods were compared with results obtained by electrophoresis. In gel electrophoresis, dilution of 1/10 was positive. Detection limits for gel electrophoresis as well as RAPID Card have been evaluated. It was shown that the Semi nested PCR method is more sensitive than conventional PCR.

Key words: HIV, Semi nested PCR.

INTRODUCTION

Over the past two decades, HIV diagnostics have been essential in detecting and monitoring infection, and continue to play a major role in saving lives throughout the world [1]. As technology evolved, screening, confirmatory, and HIV monitoring assays have been improved and offer better alternatives to address blood screening, surveillance, diagnosis, and patient management [2]. Molecular methods are critical in detecting early infection and for managing patients on anti retroviral therapy whose viral infection may become resistant to therapy. In addition, modifications to conventional methods have introduced new assays, such as sensitive/less sensitive (detuned) assays that can estimate when someone was infected, thereby providing a useful tool for epidemiologic incidence estimates and enrollment into specific intervention programmes for recently infected persons [3]. Many of the newly evolving technologies are essential for use in resource-limited countries because they can address cost issues, limited infrastructure, and a lack of formally trained personnel [4].

PCR-based techniques are increasingly being employed for the detection and diagnosis of viral diseases such as HIV infection. These techniques involve the amplification of DNA templates and can be extremely sensitive in detecting

viral levels of less than 100 copies/ml of serum35. This makes PCR particularly useful for the early detection (within two weeks of exposure) of the virus35. Techniques involving PCR are, however more complex, time consuming and expensive than serological testing [5,6,1]. A specific and rapid diagnosis such as RT-PCR assay is the most needed to minimize transmission of HIV-1 infection. Therefore, in this study we developed the RT-PCR assay that was specific against the gag gene of HIV-1. The developed RT-PCR assay was evaluated against 46 specimens that were obtained from voluntary counseling and testing for HIV (VCT) in Rumah Sakit Umum Pemerintah (RSUP) Sanglah, Bali. To get the sensitivity and specificity of RT-PCR assay, the results of assays were compared with the results of commercially serologic tests that were commonly used in Indonesia [7,8]. The RT-PCR assay could detect 21 of 26 serologic test-positive specimens and showed 19 negative results of 20 serologic test-negative specimens [9].

HIV testing has significantly improved since the first diagnostic test in 1985 [10]. Increased understanding of HIV transmission and the clinical course of the infection, together with the availability of new techniques, have changed the strategies used for HIV testing. Furthermore, now that HIV testing is recommended in the routine medical evaluation of patients, an increase in screening and the identification of HIV-infected patients is expected [11]. To adequately understand HIV testing, a review of the virus, the natural course of the infection, and the test characteristics is helpful. HIV testing has dramatically improved during the last two decades. Different HIV-testing technologies will perform differently in different settings. Selection of the most appropriate test should incorporate considerations regarding accuracy of the test, patient preferences, ease of sample collection, availability of trained personnel, availability of laboratory facilities, and prevalence of disease in the population that is being tested, among many other factors [12]. In most cases, however, an EIA can be used as a screening test, followed by one of the many confirmatory tests. Nucleic-acid amplification should be used when the detection of acute HIV infection is necessary.

MATERIALS AND METHODS

HIV ELISA: The ErbaSure HIV Gen3 is a Third generation Indirect solid phase enzyme immune sorbent assay which involves use of recombinant and synthetic antigens of HIV antigens in microplate wells. During the assay, the test specimen is first incubated with the diluent in coated microwells. The anti-HIV antibodies, if present in the specimen, bind to the antigens coated. The wells are washed to remove the unbound components after incubation. In the second incubation with the conjugate, the above bounded anti-HIV antibodies are detected by the peroxidase conjugates [13]. Unbound conjugates are then removed by washing. The presence of the bound conjugate is shown by a blue color upon additional incubation with TMB substrate reaction is stopped by adding stop solution and absorbance are read by using a photometer. The cut-off value is calculated by the given formula and absorbance of all the specimen wells is compared with the cut-off value. Any specimen having absorbance more than the cut-off value is considered reactive.

RAPID CARD METHOD:
HIV TRI-Dot: Rapid Visual Test for Detection of Antibodies to HIV in Human Serum/Plasma. HIV Antigens for core NS3, NS4, NS5.

MOLECULAR DIAGNOSIS: Semi Nested PCR
However this rapid progress presents data analysis difficulties because studies are hard to compare as technology changes. Nonetheless, it is now clear that transmission occurs almost exclusively through blood-to-blood contact. Despite the possibility of serious health consequences, it is clear that a majority of individuals chronically infected with HIV will present with few clinically overt signs [14]. In the absence of such, they tend to be diagnosed as 'healthy carriers'. However, it is now becoming apparent that this description may not always be completely appropriate. Many HIV-infected individuals, for instance, report chronic tiredness, abdominal pain, nausea, muscle and joint pains and depression in the absence of biochemical abnormalities orcirrhosis.

HIV Semi Nested PCR test: Two main molecular techniques exist for the quantification of HIV RNA, namely quantitative PCR methods and signal amplification techniques such as the branched DNA (bDNA) assay. Quantitative PCR utilizes competitive RT-PCR involving two simultaneous reactions and incorporating an internal control. **The HIV-1** Semi-nested PCR Kit constitutes a system for the detection of HIV-1 virus using polymerase chain reactions (PCR). The kit contains all reagents and enzymes for the specific amplification of LTR region of the HIV-1 genome.

Positive control: Each time the kit is used; positive control reaction must be included in the run. A positive result indicates that the primers for detecting the target pathogen gene worked properly in that particular experimental scenario. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false-positive results [15].

Negative control: To confirm the absence of contamination, a negative control reaction should be included every time the kit is used. For this reaction, the PCR grade water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run. If a positive result is obtained the results should be ignored and the test samples repeated. Possible sources of contamination should first be explored and removed.

RESULTS AND DISCUSSION

HIV Rapid Card: The rapid card results showed clear demarcation of the age group. Among all the age groups 31-35 was found to be significant for the HIV. The rapid card results of the HIV values were more for age group 31-35 and found to be 1.2. All the values were average of triplicates.

Table 1: HIV values of the serum from the patients
The values are triplicates of the samples

Age group	Level	Average
15-20	0.024	0.024
21-25	0.015	0.015
26-30	0.011	0.013
31-35	1.2	1.2
36-40	0.224	0.224
41-45	0.022	0.0165
46-50	0.182	0.182
51-55	0.018	0.018

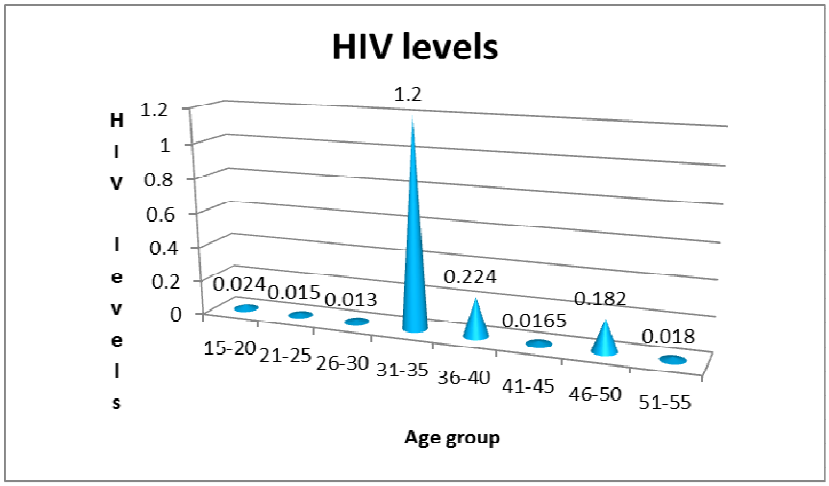

Fig 1: HIV values of different age groups. Age group of 31-35 is found to be significant
All the values are means of the triplicates

Semi Nested PCR:
Even the semi nested PCR results shown were matching the rapid card detection results. The values were found to be significant for the age group 31-35. All the values were average of triplicates.

Table 2: Comparative values of the ELISA readings and Semi nested PCR band intensities of HIV levels

Age group	HIV value	Band	Intensity
15-20	0.024	Nil	
21-25	0.015	Yes	Light
26-30	0.011	Nil	
31-35	1.2	Yes	Very Dark
36-40	0.224	Yes	Moderate
41-45	0.022	Nil	
46-50	0.182	Yes	Dark
51-55	0.018	Nil	

Semi Nested Band patterns provides a semi quantitative information: The ELISA readings shows significant values in the age group of 31-35 as well as the RAPID Cards. The Semi Nested PCR confirms of the values of the ELISA & RAPID Cards. The Bands of the gene amplified showed significant intensities in the age group 31-35 & also provides a Semi Quantitative data.

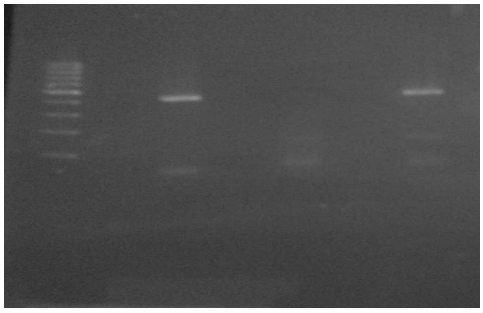

Fig 2: Agarose gel showing the amplified bands during the semi nested PCR

CONCLUSION

Polymerase Chain Reaction (PCR) is a powerful technique which is capable for amplifying little amount of target DNA into millions of identical copies. It is widely applied in molecular research and being a promising approach for routine diagnostic laboratory. However, the high amplification power in PCR also generates a potential risk for false-positive PCR result. In general laboratory practice, the specimens, other starting DNA material, could be contaminated by small aerosol in the environment present in the PCR product. Even little amount of PCR product contains many copies of target DNA and is able to generate a false-positive result. The cross-contamination problem was also reported in several laboratories.

Though Real Time PCR is much sensitive than ELISA or Conventional PCR, it is costly & not so affordable by many people. Thus the Semi Quantitative Semi Nested PCR can match the sensitivity of Real Time PCR to a considerable extent & is also cost effective.

REFERENCES

[1] Barin F, M'Boup S, Denis F, et al. *Lancet.* **1985**;2:1387-1389.
[2] Berrios DC, et al. *Arch Pathol Lab Med*. **1995** Feb;119(2):139-141.
[3] Bonhoeffer S. *AIDS Patient Care STDs.* **1998**;12:769-774.
[4] Busch MP, Lee LL, Stratten GA, et al. *Transfusion.* **1995**;35:91-97.
[5] Carpenter CC, Cooper DA, Fischl MA, et al. *JAMA.* **2000**;283:381-390.
[6] De Saussure P, Yerly S, Tullen E, et al. *Transfusion.* **1993**;33:164-167.
[7] Delaney KP, et al. *AIDS*. **2006** Aug 1;20(12):1655-1660.
[8] Durant J, Clevenbergh P, Halfon P, et al. *Lancet.* **1999**;353:2195-2199.
[9] Gaines et al. *Lancet*. **1987** May 30;1(8544):1249-1253.
[10] Hirsch MS, Brun-Vezinet F, D'Aquila RT, et al. *JAMA.* **2000**;283:2417-2425.
[11] Hollinger FB, Bremer JW, Myers LE, et al. *J Clin Microbiol.* **1992**;30:1787-1794.

[12] Jay A. Levy , Myron S. Cohen, Nick Hellmann,Kevin DeCock and Joep Lange, The spread, treatment, and prevention of HIV-1: evolution of a global pandemic ,April 1st,**2008**
[13] Mellors J, Munoz AM, Giorgi JV. *Ann Intern Med.* **1997**;126:946-954.
[14] Mylonakis E, Paliou M, Greenbough TC, Flaningan TP, Letvin NL, Rich JD. *Arch Intern Med.* **2000**;160:2386-8.
[15] O'Brien WA, Hartigan PM, Martin D, et al, *N Engl J Med.* **1996**;334:426-431.