## RESEARCH NOTE

# EVALUATION OF A NEW FOURTH-GENERATION MICROWELL ENZYME-LINKED IMMUNOSORBENT ASSAY FOR DETECTION OF HIV-1 SUBTYPE B AND E ANTIBODIES

Penprapa Chanbancherd[1], Nukool Limpairojn[1], Mark S de Souza[2,3], Achara Jugsudee[1], Pitak Julananto[1], Piched Tienamporn[1], Weerachai Leucha[1], Chatrachai Tasaniyananda[1] and Arthur E Brown[2]

[1]Army Institute of Pathology, Bangkok, Thailand; [2]Armed Forces Research Institute for Medical Sciences, Bangkok, Thailand; [3]Henry M Jackson Foundation for the Advancement of Military Medicine, Rockville, Maryland, USA

**Abstract.** The recent fourth-generation enzyme-immunoassays have been used to increase the sensitivity for detecting HIV-1 antibodies and reduce the window period of HIV infection. The HIV antigens utilized in those assays were prepared from HIV-1 clade B which is different from HIV-1 subtypes circulating in Thailand. We evaluated 323 HIV-1 seropositives either B or E subtype to determine whether they were detected with the new combined anti-HIV and the p24 Ag assay. Under evaluation we found that this enzyme immunoassay manufactured by Organon Teknika showed the high sensitivity and specificity with a greater delta ($\partial$) value with B than E subtypes samples (+15.29 *vs* +5.73).

Currently, the third-generation sandwich enzyme-linked immunosorbent assays (ELISAs) are considered to be the most sensitive of test kits for detecting HIV-1 antibodies, especially in the first weeks-months of infection (Zaaijer *et al*, 1992). High sensitivity is required for protection of the blood supply for transfusion. However, in order to reduce the window period (time from infection to seroconversion), screening for p24 HIV antigen (Ag) has been introduced in some blood banks. This additional testing is not done worldwide because of the high cost per positive unit detected. Therefore, several manufacturers have sought to improve sensitivity for earlier diagnosis of HIV infection through combination antigen-antibody enzyme immuno-assays (EIAs). European investigators have recently reported evaluations of two such fourth-generation assays (Gurtler *et al*, 1998; Weber *et al*, 1998). Their results supported the concept that combination of antigen and antibody detection in a single assay is a more efficient anti-HIV screening tool.

Correspondence: Penprapa Chanbancherd, Army Institute of Pathology, 315 Rajvithi Road, Bangkok 10400, Thailand.
Tel: (662) 245-8154; Fax: (662) 644-4824
E-mail: penprapac@thai.amedd.army.mil

Most screening assays available in Thailand were originally developed using the subtype B virus found in North America and Europe, whereas the most common HIV-1 subtypes found throughout Southeast Asia are Thai B (or B') and E (Expert Group of the Joint United Nations Program on HIV/AIDS, 1997). Within Thailand, a molecular epidemiology study from diverse geographic regions has shown that HIV-1 subtype E accounts for >95% of sexually transmited infections (Subbarao *et al*, 1998). It has been reported previously that differences among HIV-subtypes can affect the sensitivity of some diagnostic antibody assays (Schable *et al*, 1994; Courouce *et al*, 1999). A second consideration is that the performance measures of an assay found in one population cannot be assumed to be identical in another. Thus, to assess one new fourth-generation assay in Thailand, we conducted a validation study of

the solid-phase antigen/antibody capture ELISA. This assay utilizes HIV antigens (whole virus lysate and synthetic peptides) and anti-HIV p24 antibody. We evaluated assay performance with HIV-positive plasma of known subtype.

Three hundred and twenty-three frozen (-20ºC) plasma samples which tested positive with two registered HIV screening tests were subtyped by a modified antigen-limiting V3 PEIA, as previously described (Chanbancherd *et al*, 1999). In brief, each specimen was tested at a single dilution (1:100) in milk buffer diluent against a range of peptide concentrations: 0.5, 0.05 and 0.005 μg/ml. Two peptides used were V3-CM237 (CTRTPNNNTRKSIHLGPG KAWYTTGQIIGDIRQAH) and V3-CM242 (CTRPSNNTRTSITIGPGQVFYRTGDIIG DIRKAY), which have been previously shown to distinguish HIV-1 subtypes B and E in Thai subjects (VanCott *et al*, 1994; Artenstein *et al*, 1995). All specimens were typeable, 69 as subtype B and 254 as subtype E. These samples and 508 HIV-negative plasma from blood donors were subjected to testing by the Vironostika® HIV Uni-Form II Ag/Ab assay (Organon Teknika, Boxtel, The Netherlands) according to the manufacturer's instruction. The Vironostika® HIV Uni-Form II Ag/Ab assay is based on an antigen sandwich ELISA that utilizes HIV-1 gp160 native viral envelope, HIV-1 group O and HIV-2 synthetic peptides, as well as an anti HIV-1 p24 monoclonal antibody to detect p24 antigen. Among 69 HIV subtype B and 254 subtype E samples, all are found to be positive by the Vironotika Ag/Ab test kit, giving a sensitivity of 100% for both B and E subtypes. The specificity evaluation was performed with 508 blood donor specimens. Five samples were observed to be initially reactive but no repeated reactive were found after repeat testing, giving a specificity of 99% for single testing and 100% for repeated testing. The delta values (∂), the ability of the assay to discriminate between negative and positive sample populations, for the anti-HIV-1 positive plasma, for subtype B, for subtype E and for blood donations are shown in Table 1. In the present study the Virinostika® HIV Uni-Form II Ag/Ab assay produced a greater ∂ value with B than E subtype samples (+15.29 *vs* +5.73). This difference in ∂ value is probably attributable to the fact that the antigen used was derived from HIV-1 subtype B virus. The assay may perform better with B-positive than E-positive populations. However, this ELISA separated both positive and negative populations from the cut-off (CO) quite well. Therefore, false-positive and false-negative results should be rare.

Table 1
Calculation of sensitivity, specificity and delta values of the Virinostika® HIV Uni-Form II Ag/Ab assay.

| Test panels | Amount of positive samples/ samples tested | Delta value[a] (∂) |
|---|---|---|
| HIV+, subtype B | 69/69 | +15.29 |
| HIV+, subtype E | 254/254 | +5.73 |
| HIV+, both subtypes | 323/323 | +6.49 |
| HIV- | 0/508 | -3.77 |
| Estimated sensitivity (%) | 100 | |
| Estimated sensitivity (%) | 100[b] | |

[a]The ∂ value measures the distance of a population (positive or negative) mean from the CO in standard deviation units. It is calculated after log transformation of S/CO ratios.
[b]Initial reactor rate (IRR) = 5 (0.98%). Repeat reactor rate (RRR) = 0 (0%).

In summary, the performance of the Virinostika® HIV Uni-Form II Ag/Ab assay was equivalent to or better than other anti-HIV ELISAs currently registered for blood donor screening in Thailand. The test used less sample volume (50 µl) and less time (90 *vs* 120 minutes) to perform test compared with the other registered combined assay, Enzymun-Test® HIV Combi (Roche Diagnostics GmbH, Germany). Although the study assay combined two different test principles in one assay, the potential risk of non-specific reactivity was not observed. The new combined anti-HIV and p24 Ag assay under evaluation demonstrated the ability to detect HIV-1 antibodies from viral infections of HIV-1 group M subtypes Thai B and E. However, early or recent HIV-1 infected specimens (antibody negative) of both subtypes must be studied to fully assess the performance characteristics of this assay. Only with such data could decisions be made as to whether the combined assay has an efficacy superior to the separate antibody and antigen assays.

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