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 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 (Δ) value with B than E subtypes samples (+15.29 vs +5.73).

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
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 (-20°C) 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 (CRIPTNNTKSLGKVQKTYTTGQRIGDIRQ) and V3-CM242 (CTRPSNTRTSITGPGQVFYRTGDIIGDINK), 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 Vironostika 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 (δ), 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 Vironostika® HIV Uni-Form II Ag/Ab assay produced a greater δ value with B than E subtype samples (+15.29 vs +5.73). This difference in δ 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 Vironostika® HIV Uni-Form II Ag/Ab assay.

<table>
<thead>
<tr>
<th>Test panels</th>
<th>Amount of positive samples/samples tested</th>
<th>Delta value δ</th>
<th>HIV+, subtype B</th>
<th>69/69</th>
<th>+15.29</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV+, subtype E</td>
<td></td>
<td></td>
<td>HIV+, both subtypes</td>
<td>323/323</td>
<td>+6.49</td>
</tr>
<tr>
<td>HIV-</td>
<td></td>
<td></td>
<td></td>
<td>0/508</td>
<td>-3.77</td>
</tr>
<tr>
<td>Estimated sensitivity (%)</td>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Estimated sensitivity (%)</td>
<td></td>
<td></td>
<td></td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>The δ 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.

<sup>b</sup>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.

REFERENCES


