Evaluation of a 2-Minute Anti-Human Immunodeficiency Virus (HIV) Test Using the Autologous Erythrocyte Agglutination Technique with Populations Differing in HIV Prevalence

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A total of 1,800 blood specimens (1,000 from healthy blood donors, 300 from patients with sexually transmitted diseases, and 500 from intravenous drug users) were simultaneously tested with anti-human immunodeficiency virus enzyme-linked immunosorbent assay (ELISA) kits and a newly developed 2-min test for anti-human immunodeficiency virus based on the principle of autologous erythrocyte agglutination (AGEN Biomedical Limited). We found that AGEN's rapid test was as sensitive and specific as the other ELISA kits.

Because of the serious medical and psychosocial impacts of human immunodeficiency virus (HIV) infection, a sensitive and specific test is essential to distinguish those who are infected from those who are not infected. This can be accomplished either by antigen (HIV) or antibody (anti-HIV) detection or identification (1, 9, 12). Most of the screening assays are based on enzyme-linked immunosorbent assay (ELISA), which takes as long as 2 h to be completed and thus is not practical for emergency screening (4). Therefore, many simple and rapid tests such as particle agglutination, hemagglutination, dot immunoassay, and dot enzyme immunoassay have been developed (2, 3, 6, 11, 13). The most novel assay among these is an autologous erythrocyte agglutination test (AGEN Biomedical Limited, Brisbane, Queensland, Australia), which does not require the separation of serum or plasma and the results of which can be read within 2 min (8) without sophisticated equipment. The test takes advantage of a bivalent reagent that is a chemical conjugate of a monoclonal antibody to erythrocyte surface antigens (which by itself does not cause agglutination of erythrocytes) and a synthetic gp41 peptide (7, 10, 14). The antibody to HIV type 1, if present in the blood sample, will bind to the peptide-antigen conjugate on the erythrocytes, causing cross-linking between erythrocytes that results in visible hemagglutination. The test has been extensively studied in three Australian blood banks (n = 5,160) and two Australian hospital clinics (n = 419); a specificity and a sensitivity of 99.7 and 100%, respectively, were found. A comparative study of this newly developed autologous erythrocyte agglutination test and the conventional commercial anti-HIV test kits was undertaken in three different laboratories serving three different patient populations in Thailand.

Three different groups of subjects were included in this study, namely, healthy blood donors from the National Blood Center, Thai Red Cross Society, Bangkok, Thailand (n = 1,000); intravenous drug users (IVDU) from Thanarak Hospital, Pathumthani Province, Thailand (n = 500); and patients with sexually transmitted diseases (STD) from the Venerable Disease Control Division, Department of Communicable Disease Control, Ministry of Public Health, Bangkok, Thailand (n = 300).

Five milliliters of blood was taken from each subject by venipuncture and divided equally into two tubes. One tube contained citrated blood for the anti-HIV autologous erythrocyte agglutination test, and the other contained clotted blood for anti-HIV ELISA. The two tests were performed independently at the laboratory where the specimens were obtained within 24 h of specimen collection.

The autologous erythrocyte agglutination test was performed with the AGEN SimpliRED HIV-1 Ab test (AGEN Biomedical Limited), lot no. 127-004, a rapid whole-blood agglutination test to detect antibodies to HIV. The essential component of this assay is the test reagent, a chemical conjugate of a monoclonal antibody which binds to erythrocyte surface antigens and a synthetic peptide antigen derived from the gp41 sequence of HIV type 1 envelope glycoprotein. A visible agglutination is caused by cross-linking between a monoclonal antibody-coated erythrocyte and anti-HIV in seropositive sera, as mentioned above. Briefly, 10 μl of citrated blood is mixed with 1 drop of the test reagent with a plastic stirrer on a test card for 5 to 10 s, and this is followed by a 2-min rocking by hand. Each test card is used with a single specimen, with inclusion of negative and positive controls on each card. The results for the presence or absence of hemagglutination are determined by the naked eye (8). The anti-HIV ELISAs were performed with commercial kits routinely used in each participating laboratory as shown in Table 1. Any samples positive by either assay were confirmed by immunoblot (HIV type 1 Western blot) from Diagnostic Biotechnology, Singapore. An immunoblot was considered positive or negative as described elsewhere (5).

Of the 1,000 blood donor samples tested, 4 were positive by both the AGEN test and Abbott Laboratories' anti-HIV ELISA, 4 were positive by the AGEN test but negative by

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Abbott’s ELISA, and 2 were positive by Abbott’s ELISA but negative by the AGEN test (Table 1). To simplify the estimation of sensitivity and specificity of the test, any indeterminate Western blots with a p24 band were considered positive, and all others were considered negative. With these simplified criteria, the sensitivity of the AGEN whole-blood assay is 100% with 99.6% specificity (4 false positives in 1,000), compared with 100% sensitivity and 99.8% specificity for Abbott’s anti-HIV ELISA (Table 1 footnotes and Table 2). For 500 samples in the STD group, there was only one false positive by the AGEN test (1 of 262 negatives) (Table 1). For the STD group, the sensitivity and specificity of the AGEN test were 100 and 99.6%, respectively, and there was 100% sensitivity and specificity for Organon Teknika’s anti-HIV ELISA. Among the 500 samples in the IVDU group, there were found seven discrepancies between the AGEN test and Roche’s anti-HIV ELISA, i.e., samples negative by the AGEN test and positive by the ELISA (Table 1). With the same simplified immunoblot criteria cited above, the sensitivity of the AGEN test was 99.5% (1 false negative out of 209) and the specificity was 100%, while Roche’s anti-HIV ELISA had 100% sensitivity and 97.9% specificity (6 false positives out of 291) (Tables 1 and 2).

Our large-scale evaluation of the performance of the 2-min anti-HIV screening test using autologous erythrocyte agglutination assay (the AGEN test) revealed that the test was as reliable as other ELISAs for screening. The problem encountered when comparing the results of the tests concerns what is to be considered the “gold standard,” particularly when the immunoblot is indeterminate and no follow-up sera are available. In this study, if any indeterminates with a p24 band are considered positive while all other indeterminates are considered negative, the AGEN test will still have a sensitivity of 99.5 to 100% and a specificity of 99.6 to 100% (Table 2). These results are consistent with those reported from large laboratories in Australia (15). The AGEN test results are comparable to results obtained for the other three ELISA kits simultaneously evaluated. Therefore, the use of this rapid test is quite appropriate for office practice and for emergency department screening. The AGEN test would be particularly useful for emergency screening of transfused blood as well as for field serosurvey because of its rapidity (2 min), its ease and simplicity (no need for washing or any sophisticated instruments), and its applicability with unclotted whole blood (such as from a finger prick). Nevertheless, as for all other screening tests, a supplemental or confirmatory test is still needed for any positive samples.

### TABLE 1. Results of AGEN test and anti-HIV ELISAs

<table>
<thead>
<tr>
<th>ELISA manufacturer (sample group)</th>
<th>No. of samples with result:</th>
<th>Total no. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AGEN⁺/ELISA⁺</td>
<td>AGEN⁺/ELISA⁻</td>
</tr>
<tr>
<td>Abbott (blood donors)</td>
<td>4⁺</td>
<td>4⁺</td>
</tr>
<tr>
<td>Organon (STD patients)</td>
<td>38⁺</td>
<td>1⁻</td>
</tr>
<tr>
<td>Roche (IVDU)</td>
<td>206⁺</td>
<td>0</td>
</tr>
</tbody>
</table>

a Three samples were confirmed positive by Western blot, and one was indeterminate, with only a p24 band.

b All samples were confirmed negative by Western blot.

c All samples were confirmed positive by Western blot.

d Five samples were confirmed negative by Western blot, and two were indeterminate (one with only a p55 band and the other with a p24 band plus a p55 band).

e Five samples were confirmed negative by Western blot, and two were indeterminate (one with only a p55 band and the other with a p24 band plus a p55 band).

### TABLE 2. Sensitivities and specificities of AGEN test and three anti-HIV ELISAs

<table>
<thead>
<tr>
<th>Sample population</th>
<th>AGEN test</th>
<th>ELISA⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity (%)</td>
<td>Specificity (%)</td>
</tr>
<tr>
<td>Blood donors (n = 1,000)</td>
<td>100</td>
<td>99.6</td>
</tr>
<tr>
<td>STD patients (n = 300)</td>
<td>100</td>
<td>99.6</td>
</tr>
<tr>
<td>IVDU (n = 500)</td>
<td>99.5</td>
<td>100</td>
</tr>
</tbody>
</table>

a The calculation was based on the assumption that any indeterminate immunoblots with a p24 band should be considered positive whereas all other indeterminates should be considered negative.

b The prevalence of HIV infection within a given population was low for blood donors, medium for STD patients, and high for IVDU.

c ELISA kits used were that of Abbott for blood donors, that of Organon for STD patients, and that of Roche for IVDU.

### REFERENCES


