

EVALUATION OF RAPID DIAGNOSTIC TESTS FOR THE DETECTION OF HUMAN IMMUNODEFICIENCY VIRUS TYPES 1 AND 2, HEPATITIS B SURFACE ANTIGEN, AND SYPHILIS IN HO CHI MINH CITY, VIETNAM

TRUONG XUAN LIEN, NGUYEN THI KIM TIEN, G. FRASER CHANPONG, CAO THU CUC, VII THUY YEN,
R. SODERQUIST, K. LARAS, AND A. CORWIN

Pasteur Institute, Ho Chi Minh City, Vietnam; U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia

Abstract. An evaluation of three new rapid diagnostic test kits for human immunodeficiency virus types 1 and 2 (HIV-1/2), hepatitis B surface antigen (HBsAg), and syphilis involved a two-phase comparison of rapid diagnostic assays using prospectively collected from hospitals and clinics in Ho Chi Minh City, Vietnam. After specificity and sensitivity testing, three new rapid diagnostic test kits were tested in parallel with six commonly used diagnostic test kits. The Determine™ HIV-1/2 test had fewer indeterminate or equivocal results than the Capillus® HIV-1/HIV-2 or HIV Blot 2.2™ tests. However, the Determine™ HIV-1/2 test yielded one false-positive result when compared with the Serodia® HIV, HIV Blot 2.2™, and microparticle enzyme immunoassay (IMx®) HIV tests. The Serodia® HBsAg test yielded more false-negative results when compared with the Determine™ HBsAg diagnostic test kit. The results of the syphilis diagnostic tests evaluated in this clinical trial consistently agreed with those of the rapid plasma reagin test for syphilis. The Determine™ Syphilis *Treponema pallidum* (TP) test had three false-positive results compared with the Serodia® TP and the Serodia® TP•particle agglutination (PA) tests, which had two false-positive results that were confirmed as negative by an ELISA. Application of these serologic tests within this comparative evaluation framework, using the World Health Organization alternative testing strategies, proved to be an effective way to determine serostatus related to HIV, hepatitis B, and syphilis.

In many developing areas worldwide, field and clinic laboratory capabilities may be insufficient for the detection of infectious agents for definitive clinical diagnostic purposes. The absence of simple, rapid diagnostic testing methods for sexually transmitted diseases (STDs) and hepatitis has significantly hampered public health efforts to retard the spread of these diseases. The inability to provide tests for quick recognition of human immunodeficiency virus (HIV), hepatitis B, and syphilis has allowed infected individuals to unknowingly spread the disease through sexual contacts, blood donations, and intravenous needle sharing. In cities throughout Asia, current laboratory evaluation of blood specimens may preclude case follow-up and counseling due to a long time lag between initial sample collection and conventional test completion. High-risk populations typically seek treatment during clinic visits in association with acute episodes and are not likely to return a second time for test results.

Diagnostic technology is adapting itself for application in developing countries. Advancements in the laboratory diagnosis of HIV/acquired immunodeficiency syndrome (AIDS), hepatitis B, and syphilis have considered the following conditions, including: 1) speed of results; 2) test validity and accuracy; 3) minimal specimen requirement; 4) variable type of specimen, including whole blood; 5) ease of test kit use, with few requirements for specialized laboratory equipment; and 6) stable reagents, requiring no refrigeration. These criteria for the nine diagnostic tests evaluated are listed in Tables 1, 2, and 3.

MATERIALS AND METHODS

Serologic methodologies for 14 test methods were evaluated for human immunodeficiency virus types 1 and 2 (HIV-1/2), hepatitis B surface antigen (HBsAg), and syphilis (*Treponema pallidum*). The primary purpose of this investigation was to complete a comparative evaluation of three new rapid diagnostic test kits. A total of 710 patients from

the Pasteur Institute, Tuberculosis Hospital, Tropical Disease Center, STD Center, and Tudu Obstetrical Hospital in Ho Chi Minh City, Vietnam during October–December 1997 were included. Patient specimens were divided into four groups, including 199 samples to be tested for HIV (Group 1), 200 samples to be tested for HBsAg (Group 2), 163 samples to be tested for syphilis (Group 3), and 148 samples from patients with potentially cross-reacting blood components, of which 148 were tested for HIV and 128 were tested for HBsAg and syphilis. (Group 4) (Figure 1). Among these 710 patients, there were 562 sera and 280 duplicate whole blood and plasma specimens. Group 4 included 148 specimens from patients who were classified as individuals with potentially cross-reactive conditions or infections. These conditions included pregnancy and high-risk of STD contact or infections with tuberculosis, positivity for antibody to hepatitis A virus (HAV), HBsAg, HIV, syphilis, and malaria. Study activities were undertaken only after review and approval by the Committee for the Protection of Human Subjects at the U.S. Naval Medical Research Unit No. 2 (Jakarta, Indonesia) and the Committee for Human Use at the Pasteur Institute (Ho Chi Minh City, Vietnam).

The specimens were obtained from a diverse cross-section of volunteers through institutions cooperating with the Pasteur Institute (Ho Chi Minh City, Vietnam). Samples of sera, plasma, and whole blood were collected from high-risk volunteers, pregnant females, and patients with other known infectious diseases that potentially could interfere with serologic testing, including tuberculosis, antibody to HAV, syphilis, HIV, or malaria (Figure 1).

A 15-ml blood sample was obtained by trained personnel using the Becton-Dickinson (Rutherford, NJ) EDTA Vacutainer® blood collection system for whole blood and plasma and Vacutainer® tubes without anticoagulant for sera (Figure 1). Specimens were processed by standardized methods and tested following the manufacturer's instructions for all the diagnostic test methods. Initial specimen screening and clas-

TABLE 1
Comparison of evaluation criteria for three rapid diagnostic tests for human immunodeficiency virus 1 and 2 (HIV 1/2)*

	WHO criteria†	Determine [®] HIV 1/2	Serodia [®] HIV	Capillus [®] HIV-1/HIV-2
Speed of results	Rapid defined as <30 min	15 min	150 min (2.5 hr)	10 min
Test accuracy	Based on SEN (sensitivity) and SP (specificity)	SEN = 100% SP = 99.6%‡ (98/98) (248/249)	SEN = 100% SP = 100% (98/98) (249/249)	SEN = 100% SP = 99.6%§ (98/98) (248/249)
Minimum specimen volume	Volume of serum required	50 µl	25 µl	10 µl
Variable specimen type	Type of specimen required	Serum, plasma, or whole blood	Serum, plasma	Serum, plasma
Ease of test kit use	Ease of performance, specialized equipment requirements	Very easy: 1-step procedure¶; immunochromatographic result visually read; no specialized equipment	Easy: 4-step procedure; gelatin agglutination result visually read; specialized mixer/shaker required	Easy: 3-step procedure; latex agglutination result visually read; no specialized equipment
Stable reagents	Storage at ambient temperature or 2–8°C	Room temperature (2–30°C)	2–10°C	2–8°C

* Product registered trademark as follows: Determine[®] HIV 1/2, HBsAg Syphilis TP[®] (Abbott Laboratories); Serodia HIV[®], HBsAg, TP[®], TP-PA (Fujirebio); Capillus[®] HIV (Cambridge Biotech Limited).

† Based on World Health Organization (WHO) *Laboratory Biosafety Manual*. Geneva.¹⁶

‡ One false-positive result with a malaria patient using Determine[®] HIV 1/2 (see Tables 4 and 5).

§ One equivocal result with a malaria patient using Capillus[®] HIV-1/HIV-2.

¶ Two steps for whole blood specimens.

sification was completed at the Pasteur Institute (Ho Chi Minh City, Vietnam) by research staff assisted by researchers from the U.S. Naval Medical Research Unit No. 2 (Jakarta, Indonesia).

Test validity was measured with the following formulas. The formula used for calculating sensitivity was the ratio of the number of positive test results (a) that were true positives divided by the total number of positive results [a/(a + b)], where b = a false-positive result. The formula used to calculate specificity was the ratio of the number of negative results (d) that were true negatives divided by the total number of negative results. [d/(c + d)] where c = a false-negative result. The ratio of the number of patients with a disease divided by the number of all positive diagnostic test results, including false-positive results, is the positive predictive value (PV+). The formula used for calculating the PV+ was

the ratio of the number of positive test results (a) that were true positives divided by the total number of positive test results [a/(a + c)]. The negative predictive value (PV-) is a percentage based on the ratio of the number of patients without a disease and total number of negative diagnostic test results, including false-negative results. The formula used for calculating the PV- was the ratio of the number of negative test results (d) that were true negatives divided by the total number of negative test results [d/(b + d)].

The clinical trial included the examination of 347 patient specimens for HIV, 328 for hepatitis, and 291 for syphilis. Initially, the rapid diagnostic laboratory tests were evaluated based on a comparison with confirmatory tests considered to be the gold standard. The investigation evaluated the test accuracy of three new diagnostic assays for the identification of HIV, HBsAg, and syphilis based on sensitivity and spec-

TABLE 2
Comparison of evaluation criteria for three rapid diagnostic tests for hepatitis B surface antigen (HBsAg)*

	WHO criteria†	Determine [®] HBsAg	Serodia [®] HBsAg	Dainascree [®] HBsAg
Speed of results	Rapid defined as < 30 min	15 min	90 min†	15 min
Test accuracy	Based on SEN (sensitivity) and SP (specificity)	SEN = 100.0% SP = 100.0% (117/117) (211/211)	SEN = 95.7%§ SP = 100.0% (112/117) (211/211)	SEN = 100.0% SP = 100.0% (117/117) (211/211)
Minimum specimen volume	Volume of serum required	50 µl	>5–25 µl	25 µl
Variable specimen type	Type of specimen required	Serum, plasma, or whole blood	Serum, plasma	Serum, plasma
Ease of test kit use	Ease of performance, specialized equipment requirements	Very easy: 1-step procedure¶; immunochromatographic result visually read; no specialized equipment	Easy: 5-step procedure; reverse passive hemagglutination; specialized mixer/shaker required	Easy: 4-step procedure; immunochromatographic no specialized equipment
Stable reagents	Storage at ambient temperature or 2–8°C	Room temperature (2–30°C)	2–10°C	2–8°C

* Product registered trademark as follows: Determine[®] HBsAg (Abbott Laboratories); Serodia[®] HBsAg, (Fujirebio); Dainascree[®] HBsAg (Abbott Laboratories).

† Based on World Health Organization (WHO) *Laboratory Biosafety Manual*. Geneva.¹⁶

‡ Includes 30-min reagent preparation time.

§ Three false negatives, 1 equivocal, and 1 indeterminate from confirmed positive specimens with Serodia[®] HBsAg.

¶ Two steps for whole blood specimens.

TABLE 3
Comparison of evaluation criteria for three rapid diagnostic tests for syphilis*

	WHO criteria†	Determine™ Syphilis TP	Serodia® TP	Serodia® TP-PA
Speed of results	Rapid defined as <30 min	15 min	150 min‡ (2.5 hr)	120 min (2 hr)
Test accuracy	Based on SEN (sensitivity) and SP (specificity)	SEN§ 100.0% (72/72) SP§ 98.6% (216/219)	SEN§ 98.6% (71/72) SP§ 99.1% (217/219)	SEN§ 98.6% (71/72) SP§ 99.1% (217/219)
Minimum specimen volume	Volume of serum required	50 µl	25 µl	25 µl
Variable specimen type	Type of specimen required	Serum, plasma, or whole blood	Serum only	Serum, plasma
Ease of test kit use	Ease of performance, specialized equipment requirements	Very easy: 1-step procedures¶; immunochromatographic result visually read; no specialized equipment	Easy: 4-step procedure; hemagglutination; specialized mixer/shaker required	Easy: 4-step procedure; passive particle agglutination; specialized equipment
Stable reagents	Storage at ambient temperature or 2–8°C	Room temperature (2–30°C)	2–10°C	2–10°C

* Product registered trademark as follows: Determine™ Syphilis TP (Abbott laboratories); Serodia® TP, TP-PA (Fujirebio).

† Based on World Health Organization (WHO) *Laboratory Biosafety Manual*. Geneva.¹⁶

‡ Includes 30-min reagent preparation time.

§ The specificity and sensitivity are based on the rapid plasma reagin, not the fluorescent treponemal antibody absorption test kit.

¶ Two steps for whole blood specimens.

ificity measures. Second, these three assay methodologies were compared with six commonly used diagnostic test kits in a clinical trial. The clinical trial compared the different diagnostic laboratory tests by simultaneously examining known positive and negative specimens. Special consideration was given to specimens from pregnant females, as well as from malaria and tuberculosis patients. Whole blood, plasma, and serum specimens were tested with the three Determine™ (Abbott Laboratories, Abbott Park, IL) test kits. Because of limitations in the diagnostic test technologies, the Serodia® HIV, Capillus® HIV-1/HIV-2, Serodia® HBsAg, Dainascreen™ HBsAg, Serodia® TP, and Serodia® TP•PA tests were evaluated only with plasma and serum.

Serostatus, whether a specimen was seropositive or seronegative, was based on classification by standardized methods reported in the literature to be confirmatory tests of high reliability. These standard methods were considered to be the gold standard for a definitive classification of patient serostatus for this evaluation. A description of each testing method follows.

Human immunodeficiency virus types 1/2. The gold standard serologic tests for HIV are the enzyme immunoassay (EIA) and the Western blot.^{1–5} The Determine™ HIV-1/2 (Abbott Laboratories), Serodia® HIV (Fujirebio, Tokyo, Japan), and Capillus® HIV-1/HIV-2 (Cambridge Biotech, Ltd., Galway, Ireland) product sensitivity and specificity calculations were based on the comparison of test results with an indirect enzyme immunoassay (Genscreen® HIV-1/2; Sanofi, Tokyo, Japan) and a Western blot test (New Lav Blot I for HIV; Sanofi Diagnostic Pasteur, Paris, France) (Figure 2). A total of 347 specimens that were classified as seropositive or seronegative by these gold standard serologic tests. Positive and discordant samples were retested by an EIA (OTC Vironostika® HIV Uniform II plus O™; Organon Teknika, Boxtel, The Netherlands) and a microparticle enzyme immunoassay (IMx®) HIV test (Abbott Laboratories). Whole blood, plasma, and serum specimens from 182 volunteers were tested with the Determine™ HIV-1/2 test method. Specimens with indeterminate results were retested in

duplicate by the three rapid test kits, with confirmatory testing by Western blot (Diagnostic Biotechnology HIV Blot 2.2™; Genelabs Diagnostics PTE, Ltd., Singapore).

After initial classification, the specimens were examined for seroreactivity in the Determine™ HIV, Serodia® HIV, and Capillus® HIV-1/HIV-2 diagnostic test kits. The comparison between these three diagnostic tests forms the foundation of this comparative evaluation of rapid diagnostic techniques. The Determine™ HIV-1/2 test is an immunochromatographic test. Antibodies to HIV-1 or HIV-2 present in the serum bind to an antigen-selenium colloid that is captured by immobilized antigen and forms a red line on the test strip. The Serodia® HIV and Capillus® HIV-1/HIV-2 tests are based on agglutination of antigen-coated gelatin or latex particles, respectively. These two diagnostic test methods are visually interpreted, unaided by specialized equipment. The Serodia® HIV test requires the use of a plate mixer/viewer.

Hepatitis B surface antigen. The testing of sera for HBsAg was carried out at the Pasteur Institute using a standard EIA (Monolisa® Ag HBs; Sanofi) (Figure 3). Discordant results were resolved by retesting with the IMx® HBsAg test (Abbott Laboratories). The product sensitivity and specificity calculations of the Determine™ HBsAg (Abbott Laboratories), Serodia® HBsAg (Fujirebio), and Dainascreen™ HBsAg (Abbott Laboratories) tests were based on a comparison with the Monolisa® Ag HBs EIA for HBsAg. The 328 specimens were then examined for seroreactivity with the Determine™ HBsAg, Dainascreen™ HBsAg, and Serodia® HBsAg diagnostic test kits. Whole blood, plasma, and serum specimens from 184 volunteers were tested with the Determine™ test method. The Determine™ HBsAg and Dainascreen™ HBsAg test are immunochromatographic tests. Hepatitis B surface antigen binds to an antibody-selenium colloid that is captured by immobilized antigen and forms a red line or precipitate on the nitrocellulose strip or test pad, respectively. The Serodia® HBsAg diagnostic test is a reverse passive hemagglutination of erythrocytes coated with anti-HBsAg immunoglobulin. Two of these diagnostic tests are visually interpreted, unaided by specialized equip-

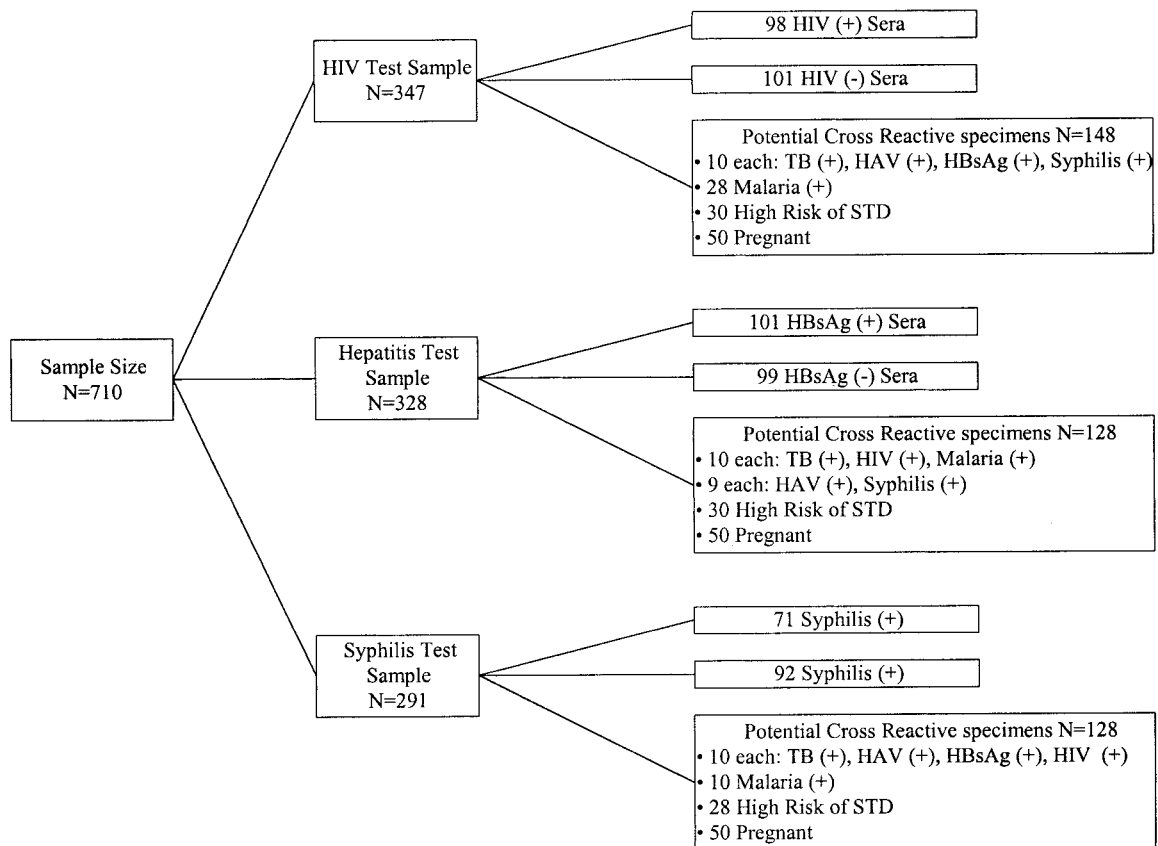


FIGURE 1. Three evaluation groups by patient type and sample size ($n = 710$). HIV = human immunodeficiency virus; TB = tuberculosis; HAV = hepatitis A virus; HBsAg hepatitis B surface antigen. STD = sexually transmitted disease.

ment. The Serodia® HBsAg test requires the use of a plate mixer/viewer.

Syphilis. The *Treponema pallidum* microhemagglutination assay (TPHA)® (Fujirebio) and rapid plasma reagin (Venereal Disease Research Laboratory [VDRL] Carbon Antigen® rapid plasma reagin [RPR]; Biomerieux, Marcy-l'Etoile, France) tests for syphilis were used in this evaluation to classify serostatus (Figure 4). The RPR test results were compared to those of the three rapid diagnostic techniques being evaluated. The confirmatory test for syphilis used in this evaluation was the OTC Trepanostika Microelisa™; Organon Teknika). This test is an ELISA that detects antibodies to *Treponema* and requires an ELISA reader for interpreting the results. Discordant samples were retested using the same test and the fluorescent treponemal antibody absorption (FTA-ABS) SG kit™ (Kyowa Yakuhin Kogyo, Tokyo, Japan).

For this evaluation, three diagnostic test methods were used to examine 291 patient sera for syphilis. The product sensitivity and specificity calculations of the Determine™ Syphilis *T. pallidum* (TP) (Abbott Laboratories), the Serodia® TP, and the Serodia® TP•particle agglutination (PA) (Fujirebio) tests were based on a comparison with an EIA (OTC Trepanostika Microelisa™) for syphilis. Whole blood, plasma, and serum specimens from 161 volunteers were tested with the Determine™ Syphilis TP test method. This test is an immunochromatographic test. Antibodies to *T. pallidum* bind to an antigen-selenium colloid that is captured by

immobilized antigen and forms a red line on the test strip. The Serodia® TP and Serodia® TP•PA diagnostic tests for syphilis are based on the use of agglutination of *T. pallidum*-coated erythrocytes or latex particles, respectively. The Determine™ diagnostic test method requires no specialized equipment because the results are interpreted visually. The Serodia® TP and TP•PA tests require the use of a plate mixer/viewer.

RESULTS

Serologic test results were divided into positive, negative, and equivocal/indeterminate findings, as recommend by the manufacturers' of the diagnostic tests. The following information summarizes the comparisons between three test methods for each of the patient conditions: HIV, hepatitis, and syphilis.

Human immunodeficiency virus types 1/2. The Determine™ HIV-1/2 rapid diagnostic test kit had a positive predictive value of 98.98% (98 of 99) among HIV+ patients classified as seropositive, and a negative predictive value of 100.0% (248 of 248) (Table 4). This test had a sensitivity of 100.0% (98 of 98) among the sera from HIV-1-seropositive patients (Table 1). Similar results were found for the Serodia® HIV and Capillus® HIV-1/HIV-2 tests. The PV+ of the Serodia® HIV test was 100.0% (98 of 98) and that of the Capillus® HIV-1/HIV-2 test was 98.98% (98 of 99) for sera from patients with evidence of HIV infection. The PV- of

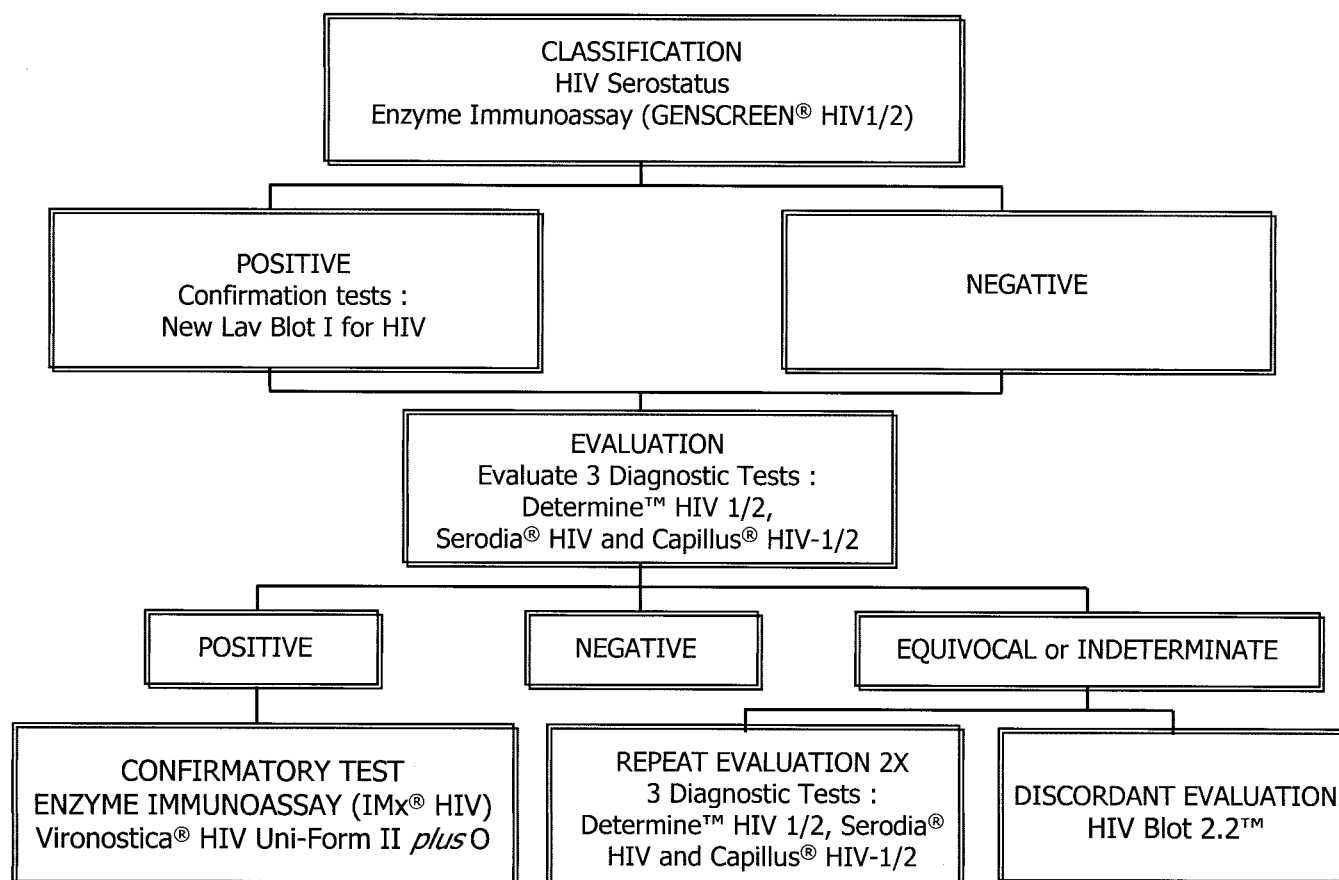


FIGURE 2. Algorithm for application of diagnostic tests for HIV. For definitions of abbreviations, see Figure 1.

the Serodia® HIV and Capillus® HIV-1/HIV-2 tests were both 100.0% for sera from patients with no evidence of HIV infection. One false-positive result was obtained with the Determine™ test. The Capillus® HIV-1/HIV-2 test yielded an equivocal test result for the same patient with malaria.

A comparison between the results of the Determine™ HIV-1/2, Serodia® HIV, and Capillus® HIV-1/HIV-2 test kits revealed an agreement of 99.7% (346 of 347) (Table 5). The discordant sample was retested using the HIV-1/HIV-2 EIA, the IMx® HIV test, and Western blotting. This sample, which was from a patient with *Plasmodium falciparum* malaria, was confirmed to be negative, yielding one false-positive result for the Determine™ HIV-1/2 test. The Serodia® HIV and Genscreen® HIV-1/2 tests correctly classified the sample as negative, while the Capillus® HIV-1/HIV-2 test yielded an equivocal result.

We examined 148 specimens for nonspecific cross-reactivity to HIV-1/2 virus among pregnant women and patients with malaria, tuberculosis, syphilis, antibody to HAV, or positivity for HBsAg who were considered to be at high risk of acquiring an STD. These specimens included serum, plasma, and whole blood for the Determine™ HIV-1/2 test. Only serum and plasma were examined by the Serodia® HIV and Capillus® HIV-1/HIV-2 tests. Whole blood, plasma, and serum samples analyzed by the Determine™ HIV-1/2 test demonstrated 100% agreement among the 180 specimens.

Hepatitis B surface antigen. During the clinical trial of the three diagnostic test methods for hepatitis, the Deter-

mine™ HBsAg test showed a PV+ of 100.0% (117 of 117) for patients with hepatitis B classified by EIA as seropositive for HBsAg (Table 4). Similar results were found for with the Serodia® HBsAg and Dainascreen™ HBsAg test (PV+ = 100%). The Determine™ HBsAg test had a sensitivity of 100.0% (117 of 117) for sera from patients classified by EIA as seropositive for HBsAg (Table 6). However, the Serodia® HBsAg test showed three false-negative results, one equivocal results, and one indeterminate result from confirmed positive specimens, for an overall sensitivity of 95.7% (112 of 117). The specificities of the Determine™ HBsAg, Dainascreen™ HBsAg, and Serodia® HBsAg tests were 100.0% (211 of 211) for sera of patients classified by EIA as seronegative for HBsAg.

An evaluation of data related to the tests of agreement between the Determine™ HBsAg and Dainascreen™ HBsAg tests showed concordance of 100.0% (328 of 328). A similar test of agreement between the Determine™ HBsAg and Serodia® HBsAg tests showed a concordance of 98.5% (323 of 328). The IMx® HBsAg test was used to retest discordant samples. The five sera with discordant results between the Determine™ and Serodia® tests were shown to be correctly classified as positive by the Determine™ HBsAg, Dainascreen™ HBsAg, and IMx® HBsAg tests.

We examined specimens for nonspecific cross-reactivity to HBsAg among pregnant women and patients with malaria, tuberculosis, HIV/AIDS, syphilis, antibody to HAV, and volunteers considered being at high-risk of acquiring an STD.

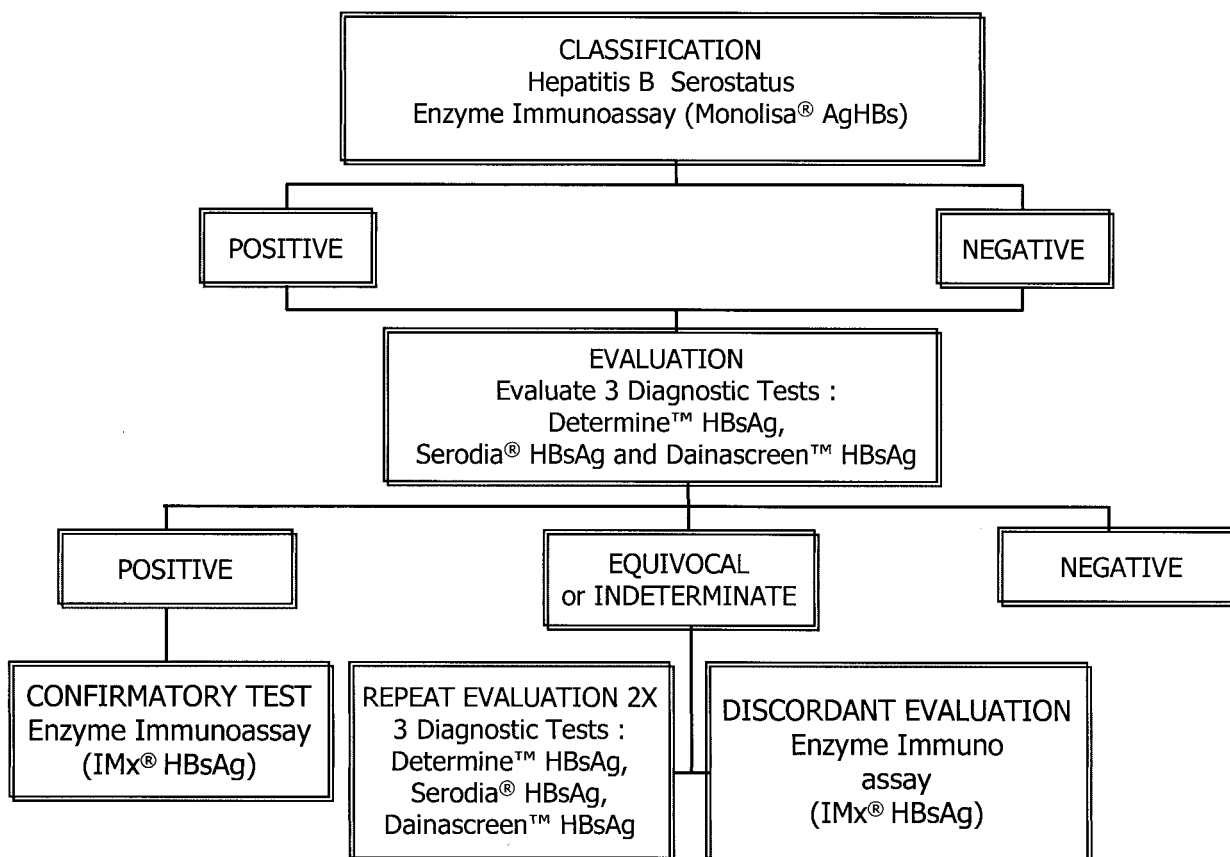


FIGURE 3. Algorithm for application of diagnostic tests for hepatitis. IMx = microparticle enzyme immunoassay. For definitions of other abbreviations, see Figure 1.

The clinical trial revealed no false-negative/positive test results among the 128 patients with potentially interfering substances in their specimen. The specimens included serum, plasma, and whole blood for the Determine™ HBsAg test and serum and plasma for the Serodia® HBsAg and Dainascreen™ HBsAg tests. There was 100% concordance between serum, plasma, and whole blood specimens.

Syphilis. The PV+ and specificity of the Determine™ Syphilis TP were not evaluated within this comparative analysis because, unfortunately, FTA-ABS testing providing a gold standard was not performed. There was 99.3% (289 of 291) agreement between the Determine™ Syphilis TP, the Serodia® TP, and the Serodia® TP•PA tests among the patient sample examined. There were two samples that demonstrated discordant results between serum, plasma, and whole blood specimens. Discordant samples were retested using the FTA-ABS and confirmatory testing included the OTC Trepanostika Microelisa™.

The Determine™ Syphilis TP, the Serodia® TP, and the Serodia® TP•PA showed comparable serologic results among the patient sample evaluated. The sera with potentially interfering substances included 50 patients with tuberculosis, antibodies to HAV, HBsAg, HIV, or malaria and sera, plasma, and whole blood specimens from 50 pregnant women. Of the pregnant patients, 23 (46%) were in the third trimester. We examined 128 specimens for nonspecific cross-reactivity to syphilis antigen among pregnant women and patients with malaria, tuberculosis, HIV/AIDS, syphilis, hep-

atitis, and volunteers considered to be at high-risk of acquiring an STD. One hundred twenty-eight of 130 specimens collected were examined because 2 specimens had insufficient serum for confirmatory testing. Serum, plasma, and whole blood from these specimens were tested by the Determine™ Syphilis TP test and serum and plasma were tested by the Serodia® TP/TP•PA tests. One whole blood specimen from a high-risk individual showed a negative result, although the serum, plasma, and confirmatory test results were positive. A second whole blood specimen from a high-risk individual also showed a negative result, but showed a weakly positive result with the serum and plasma and was classified as negative by the confirmatory tests.

DISCUSSION

The diagnostic tests evaluated are inexpensive, easy to complete, and impose the minimum discomfort to the patient, since there a very small specimen size is required. The results of any diagnostic test must be valid, accurate, reliable, and reproducible. A diagnostic test that correctly classifies patients with a disease condition as positive, and persons without disease as negative, is considered a valid and accurate test. Test sensitivity and specificity is one way to report validity that measures the degree to which those with the disease have a positive diagnostic test result. Alternatively, the PV+ and PV- report the degree in which a test actually predicts the presence or absence of disease. The

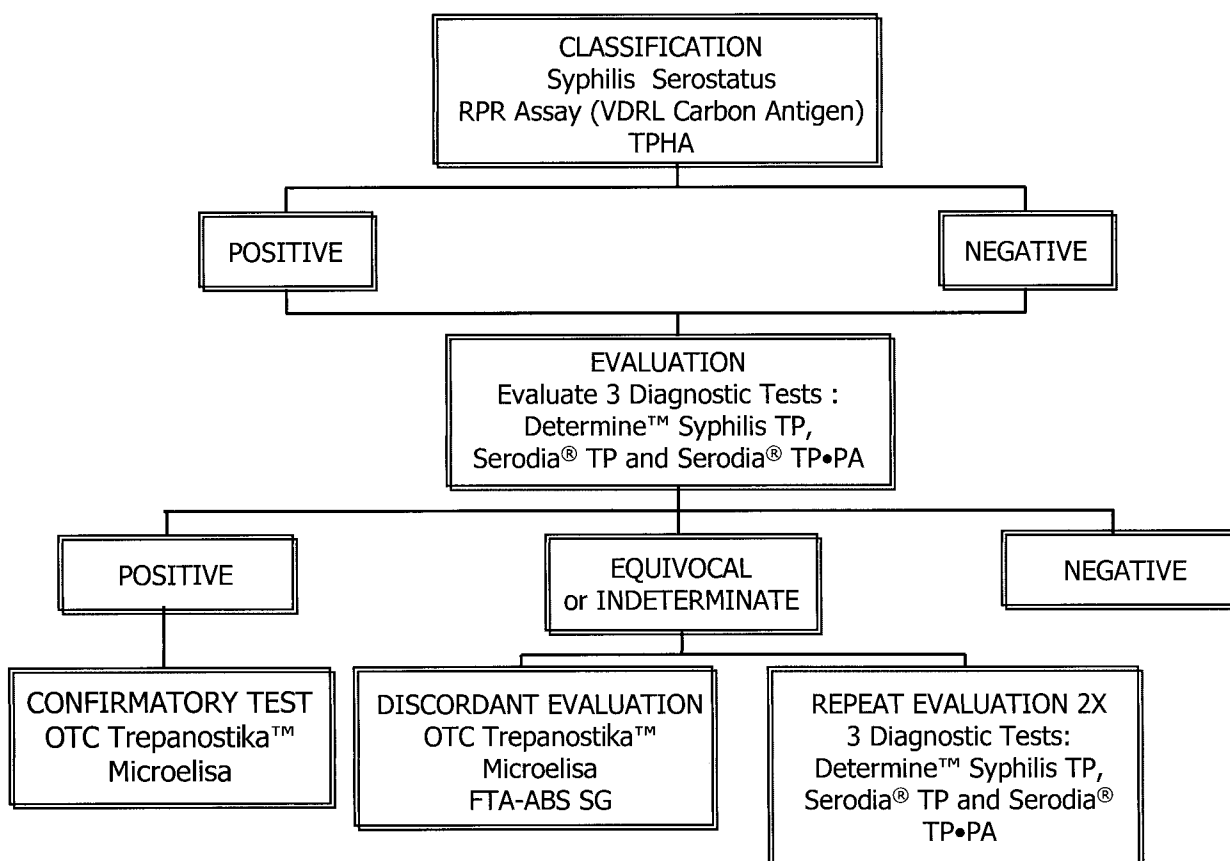


FIGURE 4. Algorithm for application of diagnostic tests for syphilis. RPR = rapid plasma reagin; VDRL = Venereal Disease Research Laboratory; TP = *Treponema pallidum*; HA = microhemagglutination; PA = particle agglutination; FTA-ABS = fluorescent treponemal antibody absorption SG kit.

PV+ and PV- are commonly used to decide whether to apply a given diagnostic test, since the degree to which a test allows for the early identification of disease is better than no treatment or delayed therapy.

An important consideration of definitive laboratory diagnosis also relates to controlling for false-positive and false-negative results. There is a potential for false readings related to other conditions, such as malaria for HIV screening and pregnancy or tuberculosis for syphilis screening.⁶⁻⁹ The ability of a diagnostic test to correctly classify patients with clinical disease as positive, and persons without disease as negative, is a measure of the validity of a specific test. Test validity is the difference between the measured effect and the true effect. Determination of test validity is a function of evaluating the specificity and sensitivity of laboratory

tests. Sensitivity reflects the ability of a test to correctly classify those who have the disease, or true positives, among those who test positive.^{10,11} Sensitivity is reported as a percentage based on the ratio of test positives and true positives, including false-negative results. Specificity refers to the ability of a test to distinguish those who do not have the infection, or true negatives. Specificity is reported as a percentage based on the ratio of test negatives and true negatives, including false-positive results.

Of particular concern to investigators was the potential for nonspecific reactivity that can occur on Western blots with HIV-negative sera, causing indeterminate results.¹²⁻¹⁴ As with all EIA techniques, there is a hazard of false-positive results from lipemic samples.⁹ Past research has demonstrated that false-positive serologic test results have occurred

TABLE 4

Comparison of Dainabot human immunodeficiency virus 1 and 2 (HIV 1/2), Serodia® HIV, and Capillus® HIV-1/HIV-2 diagnostic tests for HIV (n = 347)

EIA*	Determine™ HIV 1/2				Serodia® HIV				Capillus® HIV 1/2			
	Positive	Negative	Equivocal	Indeterminant	Positive	Negative	Equivocal	Indeterminant	Positive	Negative	Equivocal	Indeterminant
Positive†	98	0	0	0	98	0	0	0	98	0	0	0
Negative	1‡	248	0	0	0	249	0	0	0	248	1§	0

* Genscreen® and Abbott enzyme immunoassay (EIA) HIV® used as a gold standard confirmatory test.

† Genelabs® HIV Blot 2.2® and Abbot HIV-1/HIV-2 EIA® used for discordant samples.

‡ Malaria patient (M1) false-positive test result for Determine™ HIV 1/2 confirmatory test.

§ Malaria patient (M1) classified as equivocal by Capillus® HIV; indeterminate by HIV Blot 2.2.

TABLE 5

Comparison of Determine™ hepatitis B surface antigen (HBsAg), Serodia® HBsAg, and Dainascreen™ HBsAg diagnostic tests for HBsAg (n = 328)

Monolisa® Ag HBs*	Determine™ HBsAg™			Serodia® HBsAg™				Dainascreen™ HBsAg		
	Positive	Negative	Equivocal	Positive	Negative	Equivocal	Indeterminant	Positive	Negative	Equivocal
Positive	117	0	0	112	3†	1‡	1§	117	0	0
Negative	0	211	0	0	211	0	0	0	211	0

* Monolisa® Ag HBs was used as the gold standard test; Abbott IMx HBsAg was used as a confirmatory test for discordant samples.

† Three patient sera yielded false-negative results from confirmed positive specimens with Serodia® HBsAg.

‡ Patient serum yielded an equivocal result from confirmed positive specimens with Serodia® HBsAg.

§ Patient serum yielded an indeterminate result from confirmed positive specimens with Serodia® HBsAg.

with cross-reactive antibodies or interfering substances in serum.^{7,15} It should also be noted that quantitative EIA techniques for hepatitis B virus are subject to false-positive results from patients with rheumatoid arthritis.⁹ Some screening test results, such as the RPR test results, are semi-quantitative at best. False-negative results can occur in up to 50% of patients in primary phase syphilis with the RPR and other non-specific serologic tests for syphilis. False-positive results are common among patients with lupus, rheumatoid arthritis, mononucleosis, hepatitis, and patients experiencing globulin abnormalities associated with pregnancy. Sera that test positive by the RPR test are generally subjected to a confirmatory test.

Of the three diagnostic tests for HIV, the Determine™ HIV-1/2 test had fewer indeterminate or equivocal results than the Capillus® HIV-1/HIV-2 test or the HIV Blot 2.2™. However, the Determine™ HIV-1/2 test yielded one false-positive result when compared with the Serodia® HIV, HIV Blot 2.2™, and IMx® HIV tests. In many instances, false-positive results are preferable to false-negative results when screening large groups of people. Positive serology for HIV should trigger repeat testing with alternative methods for confirmation. Whole blood, plasma, and serum samples tested demonstrated 100% agreement among the 180 specimens tested by the Determine™ HIV-1/2 test. The benefit of having the flexibility to use whole blood when testing for HIV with the Determine™ HIV-1/2 test is an important clinical breakthrough, especially for blood banks.

In the diagnostic tests for hepatitis B, the Serodia® HBsAg test yielded more false-negative results when compared with the Determine™ HBsAg diagnostic test kit. The Determine™ HBsAg test showed 100.0% concordance with the Dainascreen™ HBsAg test, the gold standard EIA, and the confirmatory test (IMx® HBsAg test). The clinical trial revealed no false-negative/positive test results among the 128 patients with potentially interfering substances in their specimen.

These specimens included serum, plasma, and whole blood for the Determine™ HBsAg test. There was 100% concordance between serum, plasma, and whole blood specimens; therefore, whole blood is equally suitable for testing with the Determine™ HBsAg test.

The diagnostic tests for syphilis evaluated in this clinical trial appeared to be in agreement with the VDRL/RPR tests. However, there were three (3 of 219 = 1.4%) false-positive results in the Determine™ Syphilis TP test from specimens that were confirmed as negative by the Trepanostika Microelisa™ compared with the two (2 of 219 = 0.9%) false-positive results obtained using the Serodia® TP and Serodia® TP•PA tests. Again, false-positive results should cause physicians to complete confirmatory testing. However, of more concern was the false-negative (1 of 72 = 1.4%) result with the Serodia® products.

There were two discordant results from the 151 samples tested for syphilis, including serum, plasma, and whole blood from high-risk volunteers with concomitant infections. These specimens were re-examined using the confirmatory test (Trepanostika Microelisa™), as well as the FTA-ABS IgG/IgM tests. The results indicated that one sample was a false-positive result by the Determine™ Syphilis TP test. Two other whole blood specimens demonstrated low-intensity (indeterminate) reactivity with the Determine™ Syphilis TP assay. Therefore, the benefit of being able to use whole blood specimens with the Determine™ Syphilis TP test must be weighed against the potential for reduced specificity when using whole blood for diagnosis of syphilis.

In summary, the three new rapid Determine™ diagnostic tests (Determine™ HIV-1/2, Determine™ HBsAg, and Determine™ Syphilis TP) evaluated proved to be accurate testing methods, based on sensitivity and specificity measures, when compared with standard clinical laboratory testing. These three tests are rapid, simple, and provided excellent screening methods, with comparable sensitivity and specific-

TABLE 6

Comparison of Dainabot and Serodia rapid diagnostic tests for syphilis (n = 291)

VDRL Carbon Antigen RPR**	Determine™ Syphilis TP			Serodia® TP			Serodia® TP-PA		
	Positive	Negative	Equivocal	Positive	Negative	Equivocal	Positive	Negative	Equivocal
Positive	72	72	0	71	1†	0	71	1†	0
Negative	219	3‡	216	2§	217	0	§	217	0
Indeterminant	0	0	0	0	0	0	0	0	0

* VDRL Carbon Antigen® RPR (Biomerieux) was used as the screening for serostatus; Trepanostika Microelisa® (OTC) and FTA-ABS (Kyowa Yakuin Kougyo) were used as the confirmatory tests for discordant samples.

† Tests revealed one false-negative results using Serodia® TP/TPPA with confirmed positive serum.

‡ Tests revealed three false-positive results using Determine™ Syphilis TP with confirmed negative sera.

§ Tests revealed two false-positive results using Serodia® TP/TPPA with confirmed negative sera.

ity to the gold standard methods. Application of these serologic tests within the comparative evaluation framework, using the alternative testing strategies of the World Health Organization, proved to be an effective way to determine serostatus related to HIV, hepatitis B, and syphilis. The Determine[®] diagnostic test kits can be easily used in small, rural laboratories for serologic screening of high-risk clients. In many instances, false-positive results are preferable to false-negative results when screening large groups of people. Positive serology triggers repeat testing with alternative methods for case confirmation. Finally, the flexibility derived from being able to use whole blood for diagnostic evaluation purposes has significant clinic implications, especially for use in field-based blood banks.

Acknowledgments: We appreciate the laboratory assistance of clinical staff members at the Pasteur Institute, Cho Quan Hospital (Center for Tropical Diseases, Pham Ngoc Thach TB and Lung Diseases Center, and Tудо Obstetrical Hospital, Ho Chi Minh City, Vietnam) for the classification of the patient sera collected for the evaluation.

Financial support: This investigation was supported in part by Dainabot Company, Limited (Minato-ku, Tokyo, Japan). Dainabot manufactures laboratory diagnostic tests, including the Determine[®] products (HIV-1/2, HBsAg, syphilis), and is a subsidiary of Abbott Laboratories (Abbott Park, IL). The authors and research staff completing the investigation have no financial interest or stock options linked to the sale of any Dainabot or Abbott Laboratory products.

Authors' addresses: Truong Xuan Lien, Nguyen Thi Kim Tien, Cao Thu Cuc, and Vii Thuy Yen, Pasteur Institute, Ho Chi Minh City, Vietnam. G. Fraser Chanpong, R. Soderquist, K. Laras, and A. Corwin, U.S. Naval Medical Research Unit 2, Box 3, Unit 8132, Jakarta, Indonesia, APO AP 96520-8132.

REFERENCES

- Gonzalez L, Boyle RW, Zhang M, Castillo J, Whittier S, Della-Latta P, Clarke LM, George JR, Fang X, Wang JG, Hosein B, Wang CY, 1997. Synthetic-peptide-based enzyme-linked immunosorbent assay for screening human serum or plasma for antibodies to human immunodeficiency virus type 1 and type 2. *Clin Diagn Lab Immunol* 4: 598-603.
- Jackson JB, Parsons JS, Nichols LS, Knoble N, Kennedy S, Piwowar EM, 1997. Detection of human immunodeficiency virus type 1 (HIV-1) antibody by Western blotting and HIV-1 DNA by PCR in patients with AIDS. *J Clin Microbiol* 35: 1118-1121.
- Porter K, Mascola J, Hupudio H, Ewing D, VanCott T, Anthony R, Corwin AL, Widodo S, Ertono S, McCutchan F, Burke D, Hayes C, Wignall S, Graham R, 1997. Genetic, antigenic, and serologic characterization of human immunodeficiency virus type 1 from Indonesia. *J Acquir Immune Defic Syndr Hum Retrovirol* 14: 1-6.
- Chan EL, Sidaway F, Horsman GB, 1996. A comparison of the Genie and Western blot assays in confirmatory testing for HIV-1 antibody. *J Med Microbiol* 44: 223-225.
- Beristain CN, Rojkin LF, Lorenzo LE, 1995. Evaluation of a dipstick method for the detection of human immunodeficiency virus infection. *J Clin Lab Anal* 9: 347-350.
- Stetler H, Grande T, Nunez C, Meza R, Terrell S, Amador L, George J, 1998. Field evaluation of rapid HIV serologic tests for screening and confirming HIV-1 infection in Honduras. *AIDS* 11: 28-33.
- Westh H, Hoffmann S, Christiansen E, Worm AM, 1996. Hepatitis B core antibody screening in a high prevalence group: comparison of three enzyme immunoassays using receiver operating characteristic analysis. *J Virol Methods* 56: 13-18.
- Callahan JD, Constantine NT, Kataaha P, Zhang X, Hyams KC, Barisal J, 1993. Second generation hepatitis C virus assays: performance when testing African sera. *J Med Virol* 41: 35-38.
- Govindarajan S, Valinluck B, Lake-Bakkar G, 1991. Evaluation of a commercial antidelata EIA kit for detection of antibodies to hepatitis delta virus. *Am J Clin Pathol* 95: 240-241.
- Kleinbaum D, Kupper L, Horganstem H, 1982. *Epidemiologic Research*, New York: Norstand Reinhold, 221.
- Weiss N, 1998. *Clinical Epidemiology*. Rothman K, Greenland S, eds. *Modern Epidemiology*. Philadelphia: Lippincott-Raven Publishers, 519-528.
- Gallo D, George JR, Fitch JH, Goldstein AS, Hindahl MS, 1997. Evaluation of a system using oral mucosal transudate for HIV-1 antibody screening and confirmatory testing. *JAMA* 277: 254-258. OraSure HIV Clinical Trials Group (see comments) (published erratum appears in *JAMA* 277: 792).
- Kline RL, McNaim D, Holodniy M, Mole L, Margolis D, Blattner W, Quinn TC, 1996. Evaluation of Chiron HIV-1/HIV-2 recombinant immunoblot assay. *J Clin Microbiol* 34: 2650-2653.
- Chattopadhyaya D, Aggarwal RK, Kumari S, 1996. Further evaluation of alternative strategy for HIV testing in India. *J Commun Dis* 28: 158-162.
- van Kamp GJ, Bon GG, Verstraeten RA, Lynch D, Krikau M, Fluckiger J, Ruibal A, Kenemans P, 1996. Multicenter evaluation of the Abbott IMx CA 15-3 assay. *Clin Chem* 42: 28-33.
- World Health Organization (WHO), 1993. *Laboratory Biosafety Manual*. Geneva: World Health Organization.