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Performance and Cost-Effectiveness of a Dual Rapid Assay System for Screening and Confirmation of Human Immunodeficiency Virus Type 1 Seropositivity

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Recent studies have shown that rapid, instrument-free assays for the detection of antibody to human immunodeficiency virus (HIV) can be as sensitive and specific as enzyme-linked immunosorbent assay (ELISA) for screening of donated blood in developing countries. Currently, however, specimens which test positive on a screening assay must still be confirmed by Western blot (immunoblot), a method which is not feasible in most developing-country laboratories. We examined whether a testing hierarchy which utilizes neither conventional ELISA nor Western blot can be reliably used for screening and confirmation of HIV infection in a high-risk population. In a retrospective analysis of 3,878 specimens which were screened for antibody to HIV in Kinshasa, Zaire, we observed that a testing hierarchy consisting of duplicate HIVCHEK screening assays followed by duplicate Serodia-HIV confirmatory assays resulted in correct confirmation of all ELISA- and Western blot-positive specimens. We conclude that such a testing hierarchy can produce highly accurate results for identification of positive specimens in routine HIV testing and provides a practical alternative to conventional methods of HIV screening and confirmation.

Transfusion of unscreened blood continues to be an important route of transmission of human immunodeficiency virus (HIV) in many developing countries (3, 7). Recently, a number of instrument-free assays to detect the presence of antibody to HIV have been described, allowing minimally trained laboratory staff to rapidly and accurately screen donated blood prior to transfusion (1, 5–7, 9; P. K. Chun, E. C. Albert, R. L. Cybulski, and J. P. Galvin, Abstr. Int. Symp. African AIDS, 1987). As with enzyme-linked immunosorbent assay (ELISA) methods, however, specimens which test positive on such assays must still be confirmed by the time-consuming, costly, and highly technique-dependent method of Western blotting (immunoblotting).

We have recently shown that rapid, instrument-free assays can replace the ELISA as the primary method of blood screening for HIV in a developing country (6). For five such assays in our study, sensitivity ranged from 77.1 to 97.2% and specificity ranged from 97.9 to 99.6% compared with Western blot results. Two of the assays compared favorably with a conventional ELISA method which in our study yielded a sensitivity of 95.8% and a specificity of 99.6%. In the present paper, we analyze the data of that study from the perspective of using one rapid, instrument-free HIV screening assay to confirm the results of another and demonstrate that not only the conventional ELISA but also the Western blot can be eliminated in developing-country transfusion centers.

MATERIALS AND METHODS

Serum and collection of specimens. Serum was collected from 4,000 blood donors between November 1987 and May 1988 as part of a blood donation screening program at Mama Yemo (n = 3,090) and Ngaliema (n = 910) hospitals in Kinshasa, Zaire. Specimens were assigned randomly generated code numbers when they were obtained and were tested within 36 h of donation. When retesting or Western blotting was performed, specimens were held for as long as 1 week at 4°C without added preservatives. Six screening assays from different manufacturers, including a competitive microdilution ELISA method, were performed on each specimen. All initially positive specimens as well as every tenth negative specimen were subjected to Western blot for confirmation. The two rapid, instrument-free assays which compared most favorably with ELISA are considered in this analysis.

Laboratory methods. Serodia-HIV (Fujirebio Inc., Tokyo, Japan) is a gelatin particle agglutination assay which uses a purified lysate of HIV type 1 (HIV-1)-infected cells as antigen (9). A 25-μl portion of a test specimen was diluted 1:4 in microdilution well 1 and serially diluted twofold through microdilution wells 2 and 3. Serum in well 2 was mixed with unsensitized (negative-control) particles and serum in well 3 was mixed with HIV-1-sensitized particles by using disposable droppers. The settling pattern was observed after a 2-h incubation at ambient temperature.

HIVCHEK (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) is a second-generation immunofiltration assay which uses a recombinant HIV-1 envelope protein as antigen (Chun et al., abstract). One drop of undiluted serum

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was applied to the prewetted membrane of an assay cartridge. The membrane was washed once with buffer and once with water, 2 drops of protein A-colloidal gold conjugate was added, and a final wash was done. A dark red dot remained on the membrane if HIV-1 antibody was present in the test specimen.

Each specimen was also tested by a conventional ELISA method (Wellcozyme anti-HTLV III enzyme immunoassay; Wellcome Laboratories, Dartford, United Kingdom). Any specimen which was scored as positive on Serodia-HIV, HIVCHEK, or ELISA was retested on each of these assays and by Western blot (BioTech/Du Pont HIV Western blot kit; E. I. du Pont de Nemours & Co., Inc.). To be scored positive by Western blot, a specimen had to exhibit two or more of the bands gp160/120, gp41, and p24, the intensities of which had to be equal to or greater than that of the weakly positive control serum supplied with the kit. In addition to all initially positive specimens, every tenth negative specimen was blotted. All of these were found to be negative. In all, 1,327 specimens were Western blotted, of which 214 were positive.

Statistical comparisons. For purposes of data analysis, the following definitions and formulas were used. A specimen was scored as positive on a given screening assay only if it was repeatably reactive on duplicate assays. A true-positive (TP) specimen was positive on both screening and Western blot assays. A true-negative (TN) specimen was negative on both screening and Western blot assays. A false-positive (FP) specimen was positive on the screening assay but negative on Western blot. A false-negative (FN) specimen was negative on the screening assay but positive on Western blot. The sensitivity value is the probability that serum of an individual with antibody to HIV will be reactive in a given assay and is expressed mathematically by the formula [TP/(TP+FN)] × 100, while the specificity value is the probability that serum of an individual who does not have antibody to HIV will be nonreactive in a given assay and is expressed mathematically by the formula [TN/(TN+FP)] × 100.

RESULTS

Preliminary data. Of the 4,000 specimens tested in our original study (6), 122 were excluded from the data analysis, 117 because of incomplete data and 5 because of indeterminate Western blot patterns (HIV-specific bands which were not reproducibly as strong as the weakly positive control). Of the remaining 3,878 specimens, 214 were confirmed as positive by Western blot (a 5.5% prevalence rate). Four hundred initially negative specimens were Western blotted, none of which exhibited HIV-specific bands. Both Serodia-HIV and HIVCHEK were 97.2% sensitive compared with Western blot. Serodia-HIV was 98.2% specific, and HIVCHEK was 96.6% specific.

Having shown that results as accurate as or better than ELISA results can be obtained with a rapid, instrument-free screening assay in a developing-country transfusion center, we examined a combination of these rapid assays to determine their use for initial screening and subsequent confirmation. To address this question, we subjected our data to reanalysis. We assumed that one assay had been used to screen specimens and that a second had been used to confirm the positive results obtained with the first.

Test protocol. We developed a testing hierarchy in which an initially positive specimen would be subjected to a repeat test. If still positive, this specimen would be tested in duplicate by a confirmatory assay (Fig. 1). Other combinations are, of course, possible (duplicate screening plus single confirmation and single screening plus single confirmation, for example). We have chosen to present the data analysis for only the duplicate screening-duplicate confirmation hierarchy because this protocol provided the best accuracy. Similarly, we focused on only two (Serodia-HIV and HIVCHEK) of the five rapid assays actually evaluated in our original study, because in our study these two assays produced the highest sensitivities (97.2% for each test).

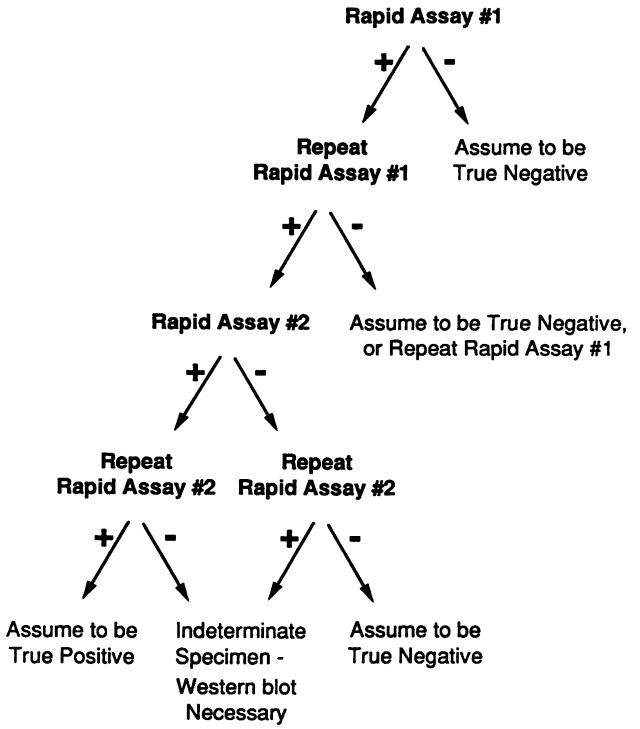

FIG. 1. Testing hierarchy used in this study in which an initially positive specimen is subjected to an additional screening test and duplicate confirmatory assays.

Sensitivity of the dual rapid assay system. The number of specimens that were repeatably positive on duplicate screening assays but could not be confirmed as positive on duplicate confirmatory assays is shown in Table 1. This included specimens which were positive on the first confirmatory assay and negative on repeat testing or vice versa (indeterminate specimens), as well as specimens which were negative on both confirmatory assays. Twenty specimens fell into this category when HIVCHEK was employed as the screen-

TABLE 1. Can one rapid assay be used to confirm another?

Screening/confirmation results[a]	No. with reference assay result[b]	
	WB^+	WB^-
$HIVCHEK^+$/$Serodia\text{-}HIV^+$	208	0
$HIVCHEK^+$/$Serodia\text{-}HIV^-$	0	11
$HIVCHEK^+$/Serodia-HIV indeterminate	6	3
$Serodia\text{-}HIV^+$/$HIVCHEK^+$	208	0
$Serodia\text{-}HIV^+$/$HIVCHEK^-$	1	61
$Serodia\text{-}HIV^+$/HIVCHEK indeterminate	5	5

[a] Indeterminate, (+, −) or (−, +) results in duplicate tests.
[b] WB, Western blot.

ing assay and Serodia-HIV was used as the confirmatory assay (Table 1); six of these, all from the indeterminate category, were true positives as judged by Western blot. When Serodia-HIV was used for screening and HIVCHEK was used for confirmation, there were 72 repeatably reactive specimens which could not be confirmed, 5 of which were true positives by Western blot. Unless demonstrated otherwise, these unconfirmed specimens would have to be subjected to additional confirmatory testing, such as Western blot.

We then examined how many of those positive specimens which could not be confirmed as positive were repeatably negative on the rapid confirmatory assay yet Western blot positive. For the combination of HIVCHEK screening and Serodia-HIV confirmation, there were no such specimens, while for the inverse combination, only one was found (Table 1). Thus, had HIVCHEK been used in duplicate as the screening assay and Serodia-HIV been used in duplicate as the confirmatory assay on this sample population, no true positives would have been missed because of incorrect confirmatory test results. If the inverse combination had been used, however, only one true positive specimen would have been missed. Thus, for this category (11 for HIVCHEK screening plus Serodia-HIV confirmation and 62 for Serodia-HIV screening plus HIVCHEK confirmation), an argument may be made that no further testing of these samples is warranted.

There were a few initially positive specimens which were negative on the first confirmatory test and positive on the repeat test or vice versa. Nine specimens fell into this indeterminate category when duplicate HIVCHEK assays were used to screen and duplicate Serodia-HIV assays were used for confirmation (Table 1). With the inverse combination of Serodia-HIV screening and HIVCHEK confirmation, there were 10 indeterminate specimens from a total of 3,878 specimens. Six of the 9 specimens in the former category and 5 of the 10 specimens in the latter category were Western blot positive. This result indicates that all indeterminate specimens should be blotted.

Specificity of the dual rapid assay system. We then asked how many specimens were repeatably positive on the screening assay and repeatably positive on the confirmatory assay and yet were Western blot negative. No false-positives were found, regardless of which assay was used for screening and which was used for confirmation (Table 1).

DISCUSSION

Adoption of routine screening of donated blood for HIV antibodies has been hampered in many developing countries by the lack of blood-banking facilities, equipment, technically trained staff, and financial resources. Yet the demand for blood in these countries is often great and seroprevalence of HIV infection in the general population can be significant, making transfusion an important route of transmission (2).

The availability of rapid, instrument-free assays for the detection of antibody to HIV may facilitate blood screening, provided that the assays are priced affordably and can be used reliably by minimally trained laboratory staff. We and others have evaluated a variety of these assays under a range of laboratory settings and found that under many conditions, performance as good as or better than that of conventional ELISA can be obtained (1, 4, 6, 8; L. Ndongala, J. Rowland, H. Francis, M. P. Duma, M. Kasali, and T. C. Quinn, Abstr. IIIrd Int. Conf. AIDS, i:166, 1987).

While a single or repeatably positive result on a rapid, instrument-free assay may be sufficient to justify eliminating a unit of blood from the supply, additional confirmatory testing is mandated if individuals are to be informed of their serological status. To date, Western blotting has constituted the major means of confirming seropositivity. Unfortunately, Western blotting is difficult to implement on a routine basis in developing countries, since it is costly, poorly standardized, and highly technique dependent. The availability of commercial Western blot kits can be expected to do little to make this technique more accessible, as the kits are too expensive for routine use in the majority of developing-country laboratories.

With this in mind, we subjected the data from our comparative evaluation of five rapid assay kits in Kinshasa, Zaire, to reanalysis from the perspective of using one rapid assay to confirm the results of another. Specifically, we wanted to know if a testing hierarchy which included neither conventional ELISA nor Western blot could ensure that no true-positive specimens were unconfirmed and that no true-negative specimens were erroneously confirmed as positive. Although we looked at multiple possible hierarchies and all possible combinations of the five assays compared in our original study, we report here data for only one possible hierarchy using only one possible pairwise combination of assays.

Had specimens been screened in duplicate by using the HIVCHEK assay and had all repeatably positive specimens been confirmed in duplicate by Serodia-HIV, no specimen would have been confirmed incorrectly, referring to Western blot as the "gold standard." The inverse combination (Serodia-HIV screening plus HIVCHEK confirmation) would have incorrectly identified only a single specimen. It should be noted that this testing hierarchy differs from that employed by blood banks in the United States, not only with respect to the testing method but with respect to two other important variables as well. First, we assumed that the confirmatory assay would be performed in duplicate, whereas in standard blood-banking practices only a single confirmatory assay is routinely done. Second, both screening assays were performed on the same specimen sample rather than on a fresh specimen, as is standard United States blood bank procedure. Therefore, an error in sample identification during screening could have been propagated. We recommend that a fresh portion of specimen be used for retesting whenever a specimen gives an initial positive result.

Another point of interest, from an economic and a public health perspective, concerned the number of specimens which were repeatably positive on duplicate screening but indeterminate on duplicate confirmatory assay, i.e., neither repeatably positive nor repeatably negative. This number is important from an economic standpoint because of the necessity of Western blotting these specimens or testing an additional specimen drawn at a later date in order to confirm the serological status of the donor and to counsel that donor appropriately. To be maximally effective, the confirmatory assay chosen should result in a minimum of indeterminate specimens. Both HIVCHEK and Serodia-HIV seem well suited as confirmatory assays, since their use resulted in identification of only 9 and 10 specimens, respectively, as indeterminate. This number may have public health ramifications as well, since in many developing countries ensuring individual patient follow-up is difficult.

HIVCHEK and Serodia-HIV are distinguished by assay principle as well as source of antigen. HIVCHEK is an immunofiltration assay which incorporates a recombinant

TABLE 2. Cost analysis of screening and confirmatory assays

Confirmation assay	Cost of assays (cost of materials) with screening assay[a]		
	HIVCHEK	Serodia-HIV	ELISA
HIVCHEK		7,334 (35.42)	NC
Serodia-HIV	8,057 (38.73)		NC
Western blot	11,673 (56.12)	11,605 (55.79)	8,580 (41.85)

[a] The total cost (in U.S. dollars) for a given combination of screening and confirmatory assays used in this study is shown. It is assumed that each screening assay is performed in duplicate, each rapid confirmatory assay is performed in duplicate, and any indeterminate specimen is Western blotted. Where Western blot is used as the confirmatory method, it assumes that only a single blot is performed. Tests and prices are HIVCHEK, $1.75; Serodia-HIV, $1.45; Wellcozyme ELISA, $1.00; Du Pont Western blot, $20.00. These prices were quoted by the manufacturers for lot sizes of 10,000 for developing-country use. Actual prices may vary. Numbers in parentheses are the costs (in U.S. dollars) of test materials per positive specimen identified using a given combination of screening plus confirmatory methods. NC, Not calculated.

peptide derived from the *env* region of the viral genome, while Serodia-HIV, which is designed primarily as a screening method, is a gelatin particle agglutination assay which uses a whole-virus lysate as antigen. A typical HIVCHEK assay can be completed in 5 min and was judged to be among the easiest assays to perform and interpret (6). However, each assay must be performed individually in its own cartridge, resulting in a limited ability to batch specimens—a disadvantage in large blood banks. The Serodia-HIV assay, which is designed primarily as a screening method, is amenable to batching. As many as 32 assays and controls can be performed simultaneously with little additional cost in time. In addition, an instrument which facilitates the collection and interpretation of data when large numbers of specimens are to be analyzed is available for purchase from the manufacturer. Nevertheless, the assay requires approximately 2 h to complete, which may not make it appropriate for use in transfusion centers where, typically, blood is not stored. Like the HIVCHEK assay, Serodia-HIV was judged easy to perform and interpret. Therefore, depending on the individual conditions and circumstances under which they are used, one assay may be more appropriate than the other for screening and/or confirmation of results. Both assays offer an additional advantage in that they provide a semipermanent record of the test result. Dried HIVCHEK cartridges and Serodia-HIV plates, when stored at ambient temperature and protected from moisture, have remained readable, in our hands, for at least 9 months.

A testing hierarchy consisting of rapid screening and confirmation assays saves time and labor and eliminates dependence on costly equipment. Nevertheless, use of a dual rapid assay system as proposed here is far from inexpensive when measured by developing-country standards. Table 2 compares the cost of materials and the cost per positive unit for five different combinations of screening and confirmatory assays identified in our study. The cost of screening and confirming any repeatably reactive specimens can vary considerably depending on the assay combination chosen. In our study of 3,878 blood donors (5.5% seroprevalence), the most expensive combination would have been HIVCHEK screening plus Western blot confirmation, at $56.12 per positive unit identified, and the least expensive would have been Serodia-HIV screening plus HIVCHEK confirmation, at $35.42 per positive unit identified.

Thus, a modest cost savings may be realized with a dual rapid assay system without compromising accuracy. The major advantages, however, are in the savings of time and labor and in the elimination of dependence on capital equipment. Accurate HIV screening and confirmation of results may now be realistically extended to transfusion centers, primary health care centers, or other areas of urgent need. However, despite these considerable advantages, the cost per HIV-positive unit identified is still high for many developing countries. A truly inexpensive, rapid HIV assay for blood is still urgently needed.

The present study is a retrospective one. However, the data clearly suggest that conventional ELISA and Western blot systems can be replaced by a combination of rapid methods in developing-country blood-screening programs with no loss in accuracy and with savings in the cost of materials. With these encouraging results in hand, prospective feasibility studies of dual rapid assay systems for blood screening and confirmation should be undertaken.

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LITERATURE CITED

1. **Carlson, J. R., J. L. Yee, and E. J. Watson-Williams.** 1987. Rapid, easy, and economical screening test for antibodies to human immunodeficiency virus. Lancet **ii**:361–362.
2. **Greenberg, A. E., P. Nguyen-Dinh, J. M. Mann, N. Kabota, R. L. Colebunders, H. Francis, T. C. Quinn, P. Baudoux, B. Lyamba, F. Davachi, J. M. Roberts, N. Kabeya, J. Curran, and C. C. Campbell.** 1988. The association between malaria, blood transfusions, and HIV seropositivity in a pediatric population in Kinshasa, Zaire. J. Am. Med. Assoc. **259**:545–549.
3. **Quinn, T. C., J. M. Mann, J. W. Curran, and P. Piot.** 1986. AIDS in Africa: an epidemiologic paradigm. Science **234**:955–963.
4. **Quinn, T. C., C. H. Riggin, R. L. Kline, H. Francis, K. Mulanga, M. G. Sension, and A. Fauci.** 1988. Rapid latex agglutination assay using recombinant envelope polypeptide for the detection of antibody to the HIV. J. Am. Med. Assoc. **260**:510–513.
5. **Riggin, C. H., G. A. Beltz, C.-H. Hung, R. M. Thorn, and D. J. Marciani.** 1987. Detection of antibodies to human immunodeficiency virus by latex agglutination with recombinant antigen. J. Clin. Microbiol. **25**:1772–1773.
6. **Spielberg, F., C. Mulanga Kabeya, R. W. Ryder, N. K. Kifuani, J. Harris, T. R. Bender, W. C. Heyward, and T. C. Quinn.** 1989. Comparative evaluation of rapid, visually read HIV antibody screening assays at Mama Yemo Hospital, Kinshasa, Zaire. Lancet **i**:580–584.
7. **Van de Perre, P., D. Munyambuga, G. Issis, J. P. Butler, D. Nzaramba, and N. Clumeck.** 1985. Antibody to HTLV-III in blood donors in Central Africa. Lancet **ii**:336–337.
8. **Van de Perre, P., D. Nzaramba, S. Allen, C. H. Riggin, S. Sprecher-Goldberger, and J.-P. Butzler.** 1988. Comparison of six serological assays for human immunodeficiency virus antibody detection in developing countries. J. Clin. Microbiol. **26**:552–556.
9. **Yoshida, T., T. Matsui, S. Kobayashi, and N. Yamamoto.** 1987. Evaluation of passive particle agglutination test for antibody to human immunodeficiency virus. J. Clin. Microbiol. **25**:1433–1437.