CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, July 2005, p. 855–860 Vol. 12, No. 7
1071-412X/05/$08.00+0 doi:10.1128/CDLI.12.7.855–860.2005

Evaluation of Rapid Prenatal Human Immunodeficiency Virus Testing in Rural Cameroon

Timothy C. Granade,[1]* Bharat S. Parekh,[1] Pius M. Tih,[2] Thomas Welty,[2] Edith Welty,[2] Marc Bulterys,[3] George Ndikintum,[2] Godlove Nkuoh,[2] and Samuel Tancho[2]

HIV Laboratory Branch, Division of HIV/AIDS Prevention, National Center for HIV, STD, and TB Prevention,[1] and Epidemiology Branch, Division of HIV/AIDS Prevention, National Center for HIV, STD and TB Prevention,[3] Centers for Disease Control and Prevention, Atlanta, GA 30333, and Cameroon Baptist Convention Health Board, P.O. Box 9, Nso, Northwest Province, Cameroon[2]

Received 3 May 2004/Returned for modification 24 June 2004/Accepted 25 April 2005

Pregnant women (n = 859) in rural Cameroonian prenatal clinics were screened by two rapid human immunodeficiency virus (HIV) antibody tests (rapid tests [RT]) (Determine and Hema-Strip) using either whole blood or plasma. One additional RT (Capillus, HIV-CHEK, or Sero-Card) was used to resolve discordant results. RT results were compared with HIV-1 enzyme immunoassay (EIA) and Western blot (WB) results of matched dried blood spots (DBS) to assess the accuracy of HIV RTs. DBS EIA/WB identified 83 HIV antibody-reactive, 763 HIV antibody-nonreactive, and 13 indeterminate specimens. RT results were evaluated in serial (two consecutive tests) or parallel (two simultaneous tests) testing algorithms. A serial algorithm using Determine and Hema-Strip yielded sensitivity and specificity results of 97.6% and 99.7%, respectively, whereas a parallel RT algorithm using Determine plus a second RT produced a sensitivity and specificity of 100% and 99.7%, respectively. HIV RTs provide excellent alternatives for identifying HIV infection, and their field performance could be monitored using DBS testing strategies.

Rapid testing (RT) methods for detecting the presence of human immunodeficiency virus (HIV) antibodies in serum or plasma were developed in the late 1980s, and improved RT assays are continually being introduced. Throughout the last decade, these assays have demonstrated excellent sensitivities and specificities, but many tests still require a laboratory to process the specimens and to maintain the integrity of the test components (5, 10, 19, 20, 26). The World Health Organization (WHO) has recommended the use of rapid HIV tests for blood safety, for surveillance, and for patient diagnosis in prenatal clinics or voluntary counseling and testing (VCT) centers (1, 3). The benefits of such testing include the ability to inform patients of their HIV serostatus at the time of testing (11, 25) and to promptly identify HIV-infected expectant mothers so that therapy for the prevention of mother-to-child transmission (PMCT) of HIV can be provided in a timely manner (16). Ideally, these tests would be used in serial or parallel testing strategies that would allow for screening and confirmation of initially reactive specimens in a single clinic visit (1).

New HIV RT that can use whole-blood specimens have been available for several years; however, little data exist about their performance in rural, resource-poor settings. To address this question, an effective method for assuring the quality of whole-blood RT results is needed. Whole blood dried on filter paper (dried blood spots [DBS]) has been applied in large-scale HIV surveillance studies for well over a decade (13). DBS can easily be collected at the same time as the whole-blood specimens for the rapid HIV assays and could be used to validate the on-site RT results. DBS are well suited for this process, since they are easy to collect, to store, and to ship to larger laboratories for supplemental testing. Several HIV antibody detection enzyme immunoassays (EIA) are approved by the United States Food and Drug Administration (FDA) for use with DBS, and other internationally available assays could be adapted for DBS testing. DBS collection and storage has been well standardized (2), and quality control and quality assurance programs have been in use for over 15 years with effective protocols readily available (14). Our study evaluated the performance of several HIV RT algorithms in rural, prenatal clinics in Northwest Province, Cameroon, and compared the RT results to those determined by EIA and Western blotting (WB) from matched DBS specimens.

MATERIALS AND METHODS

Patient counseling and specimen collection. After approval by the local Institutional Review Board, the study was conducted in Cameroon at prenatal clinics in Mbingo and Banso Baptist Hospitals and in Belo Baptist Integrated Health Center from February 2000 to November 2000. The patients received voluntary HIV counseling and testing at no charge as part of standard prenatal care. The counselor met privately with each patient, obtained a sexual history, discussed the risks and benefits of all antenatal tests, and obtained informed consent. One of the benefits of testing was the provision of nevirapine preventive treatment at no charge to HIV-infected women and to their newborns. Only 5% of the patients refused HIV testing.

The laboratory collected 2 ml of whole blood by venipuncture for the prenatal testing, which included HIV, if the patient consented. The blood was dispensed into test tubes containing EDTA and gently mixed to prevent coagulation. Five spots of whole blood (100 μl/spot) were pipetted onto blood collection cards (Schleicher and Schuell grade 903; Keene, NH) from the venipuncture collections. The spots were air dried for 3 to 4 h, put into moisture-resistant bags (Bitran series; Fisher Scientific, Atlanta, GA) with desiccant (Multisorb Technologies, Buffalo, NY), and were stored frozen at −20°C prior to shipment to the Centers for Disease Control and Prevention (CDC), Atlanta, Ga., for testing. The remaining whole blood was centrifuged for 5 min at 1,000 rpm, and the plasma was removed and stored at 4°C. Whole blood was used for the Hema-

* Corresponding author. Mailing address: Centers for Disease Control and Prevention, 1600 Clifton Rd., NE MS D-12, Atlanta, GA 30333. Phone: (404) 639-3850. Fax: (404) 639-2660. E-mail: Tgranade@cdc.gov.

TABLE 1. Characteristics of HIV test products included in the study[a]

Assay	Format	Antigen	No. tested	Use	Rapid test algorithm designation
Rapid tests					
Determine (DT)	AS	RA/SP	872	Screening or confirmation	Test 1 or Test 2
Hema-Strip (HS)	Indirect IA	SP	696	Screening or confirmation	Test 1 or Test 2
HIV-Check (HC)	Indirect IA	RA	137	Confirmation	Test 2
Sero-Card (SC)	Indirect EIA	SP	7	Testing of discordant specimens	Test 3
Capillus (CP)	AG	RA	13	Testing of discordant specimens	Test 3
Enzyme immunoassays					
rLAV	Indirect EIA	WVL/RA	879	Screening of DBS specimens	
Uniform II + O	AS	RA/SP	879	Screening of DBS specimens	
Select HIV-1/HIV-2	Indirect EIA	SP	149	Confirmation of DBS specimens	
Western immunoblot assays					
Novapath HIV-1		WVL	156	Confirmation of DBS specimens	
Miniblot HIV-1		WVL	81	Confirmation of DBS specimens	
LAV HIV-2 blot		WVL	14	Confirmation of DBS specimens	

[a] AS, antibody sandwich; RA, recombinant antigen; SP, synthetic peptide; IA, immunoassay; AG, agglutination; WVL, whole viral lysate; Miniblot, in-house miniaturized Western blot.

Strip test; plasma was used for all additional RTs. Trained laboratory staff performed the testing at all sites, and prenatal clinic nurses or counselors provided postcounseling for all of the laboratory results on the same day.

Rapid tests. RTs in the study were Determine HIV-1/2 (DT) (Abbott Laboratories, Tokyo, Japan), Hema-Strip (HS) (ChemBio, Medford, NY), Capillus HIV-1/HIV-2 (CP) (Trinity Biotech, Galway, Ireland), Sero-Card (SC) (Trinity Biotech), and HIV-CHEK (HC) (Johnson and Johnson, New Brunswick, NJ). DT and HS are lateral flow assays; CP is an agglutination assay; and SC and HC are flowthrough, immunodot assays (7). The assays were selected either for their ability to use whole-blood specimens, for their simplicity, or for their capacity to be used as supplied without additional equipment or reagents. The HS tests were performed according to the manufacturer's protocols using whole-blood specimens and were scored accordingly. The remaining tests were performed according to the kit inserts using the plasma aliquots to complete the HIV screen and to resolve discordant results.

Prospective evaluation of rapid testing algorithms. Women (n = 859) attending the three prenatal clinics were tested by two RTs in parallel using whole-blood or plasma specimens. Discordant results were resolved using a third RT. The choice of the third test was dependent on the tests available in the clinics at the time of testing. The data were analyzed as if parallel and serial testing algorithms had been used. Parallel RT algorithms compared the results of two different rapid tests. Concordant reactive and nonreactive specimens were considered definitive, while discordant results were resolved with a third test. The concordant results of two of the three assays were taken as the correct result. The serial testing algorithm considered initially nonreactive specimens as true HIV antibody-negative specimens. Reactive samples were evaluated with a second test and, if reactive, were considered as HIV antibody positive. Discordant specimens were resolved using the third RT results.

HIV-1 serologic testing. The diagnostic assays used to test the DBS, plasma, or whole-blood specimens employ different testing formats and different viral antigens and were used in different combinations to screen and then to confirm initial reactive test results (Table 1). HIV-1 reference testing was performed at the CDC, using DBS protocols approved by the U.S. Food and Drug Administration for the Genetic Systems HIV-1 rLAV (rLAV) kit (Bio-Rad Laboratories, Hercules, CA). Initially reactive specimens were retested by the same assay in duplicate, and samples that were reactive in at least two of the three tests were confirmed by Western blot (WB). WB testing was done using a miniaturized WB method previously described for DBS specimens (12) or by the Novapath HIV-1 Western blot (Bio-Rad Laboratories) as follows. Specimens were prepared by eluting a 6-mm punch of the DBS with 200 μl of 0.15 M phosphate-buffered saline plus 0.05% Tween, pH 7.4 (PBST) (Sigma Chemical Co, St. Louis, MO) for 2 h with shaking, or overnight at 4°C. One-hundred microliters of eluted DBS was added to 900 μl of the specimen diluent in the Bio-Rad WB kit, and the procedure was performed as described in the kit insert. The method was validated and quality controlled using strongly reactive, weakly reactive, and nonreactive DBS controls provided by the National Center for Environmental Health of CDC (15). The HIV–1 EIA/WB testings of DBS were used as the referent results and were performed without knowledge of the RT results.

HIV-1/HIV-2 serologic testing. All DBS were tested for HIV-1/HIV-2 antibodies using a modified procedure for the Uniform II plus O assay (Organon Teknika, Boxtel, The Netherlands). A 6-mm punch from the DBS was eluted in 200 μl of PBST overnight. After mixing, 75 μl of the eluate was mixed with 75 μl of the specimen diluent in the appropriate well, and the remainder of the assay was performed according to the manufacturer's instructions. Specimens reactive in the Uniform II assay were further tested by Select HIV-1/HIV-2 assay (Biochem Immunosystemes, Quebec, Canada) to identify the presence of HIV-1 or HIV-2 antibodies. Fifty microliters of DBS eluate was mixed with 50 μl of Select dilution buffer, and the assay was performed according to protocol. The discrimination of HIV-1 from HIV-2 specimens is based on relative signal to cutoff (S/CO) ratios which was calculated according to procedures described in the product insert. Reactive specimens for HIV-1 and HIV-2 were further tested by the HIV-1 WB as previously described or by HIV-2 WB (Genelabs Diagnostics, Singapore) as follows. One-hundred microliters of eluted DBS was added to 900 μl of the specimen diluent, and the procedure was performed as described in the kit insert. After EIA/WB testing, the specimens were classified as follows: 83 (10.1%) HIV-1 antibody reactive; 763 (88.4%) nonreactive for HIV-1 antibody; and 13 (1.5%) rLAV reactive, WB indeterminate (see Fig. 3A). The indeterminate specimens were excluded from further analysis, since the true status of the sample could not be determined.

RESULTS

Patient population. The patient population (n = 859) consisted of women of childbearing age attending the three prenatal clinics. The age of the participants ranged from 15 to 50 (mean, 26.6 years; median, 24.2 years). Twenty-two occupations were reported, with farmer (42%) and housewife (30%) being the most frequent. Eighty-two percent of the women were married, and 46 percent reported only one sexual partner over the previous 3 years. Two thirds of the women reported completing 7 years or less of formal schooling, and 7% indicated some postsecondary education.

HIV rapid testing performance. The study was done to determine the effectiveness of RTs to identify HIV-infected individuals by comparing the RT results with those determined from the matched DBS. However, in field applications many factors can impact the testing process, and, in this case, the selected RTs were not available at all sites throughout the

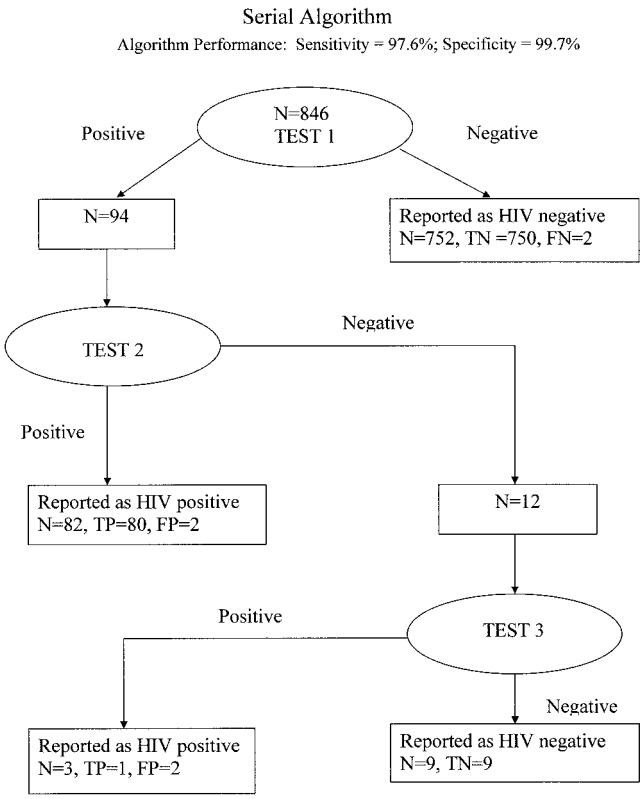

FIG. 1. Determination of HIV antibody status with rapid tests using a serial testing algorithm. N, number; TN, true negative; FN, false negative; TP, true positive; FP, false positive. TEST 1, Determine; TEST 2, Hema-Strip or HIV-CHEK; TEST 3, Capillus or Sero-Card.

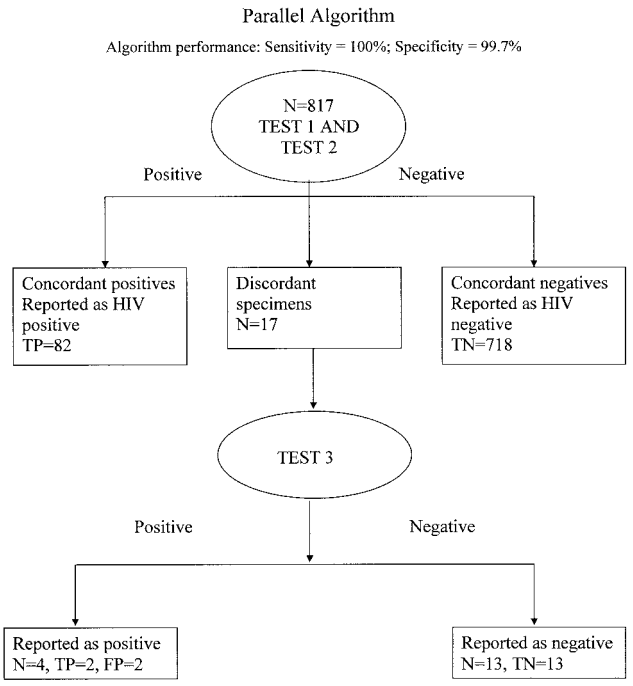

FIG. 2. Determination of HIV antibody status with rapid tests using a parallel testing algorithm. N, number; TN, true negative; FN, false negative; TP, true positive; FP, false positive. TEST 1, Determine; TEST 2, Hema-Strip or HIV-CHEK; TEST 3, Capillus or Sero-Card.

course of the study; thus, the number of tests performed with each assay varied (Table 1). The effectiveness of the RTs was evaluated using serial or parallel testing algorithms. Sensitivity and specificity of each algorithm were calculated based on the total number of RT and matched, definitive DBS results available from the three collection sites (n = 846).

Serial rapid testing algorithm. Field specimens tested by RT were evaluated in a serial testing algorithm (Fig. 1), and the data were compared to the results of the DBS reference testing. DT detected an initial 94 HIV antibody-reactive specimens and 752 antibody-negative reactions (750 true negatives, 2 false negatives). Secondary testing of the initial reactive samples was performed using HS (n = 73) or HC (n = 21). HS found 63 HIV antibody-reactive samples (61 true positives, 2 false positives). Ten specimens had discordant results between DT and HS, and these were retested by either CP or SC. One specimen was reactive by each tertiary test, but both of these were nonreactive by the reference methods (2 false positives). Of the 21 specimens tested by HC, 19 HIV antibody-positive specimens were identified (19 true positives). Two specimens were HIV antibody negative by HC. When these were tested by SC, one HIV antibody-positive and one antibody-negative specimen were identified, and these results were concordant with the DBS results. The sensitivity and specificity of the serial RT algorithm using DT as the screening assay were 97.6% and 99.7%, respectively.

Parallel testing algorithm. Results from an additional 29 women were excluded from this analysis because only DT results were available. Of the 817 specimens tested by at least two RTs, 82 were reactive by DT (Test 1) and by either HS or HC (Test 2); 718 were concordantly seronegative by two assays (Fig. 2). Retesting the discordant specimens with Test 3 (CP, 12 specimens, and SC, 5 specimens) resulted in 2 true-positive, 2 false-positive, and 13 true-negative results. In total, the parallel algorithm classified 86 specimens as HIV antibody reactive and 731 as HIV antibody negative. Eighty-four of the 86 rapid test-reactive specimens were confirmed as seropositve by DBS EIA/WB, and all of the 731 seronegative specimens were nonreactive by DBS serology (sensitivity, 100%; specificity, 99.7%). Attempts were made to contact the two women with false-positive RT results, but they were not able to be located in these remote settings that had no phones or street addresses.

EIA detection of HIV-1/HIV-2 antibodies from DBS. Since rLAV had such a high initial reactive rate and detects antibodies only to HIV-1, this assay may not be the most appropriate for screening African specimens or for quality assurance purposes. The DBS (n = 858; 1 specimen was exhausted) were also screened with the HIV Uniform II plus O (UNF) assay, which identified 138 specimens as initially reactive, including all but one of the HIV antibody-positive specimens identified by rLAV/WB (Fig. 3B). The discordant specimen was slightly below the cutoff of the UNF assay and was weakly reactive by rLAV and by WB (only gp160, p55, and p24 bands were observed). All of the UNF initially reactive specimens were further tested using the Select HIV-1/2 (SEL) assay. Results of the HIV-1 SEL confirmed 82 HIV-1 antibody-positive speci-

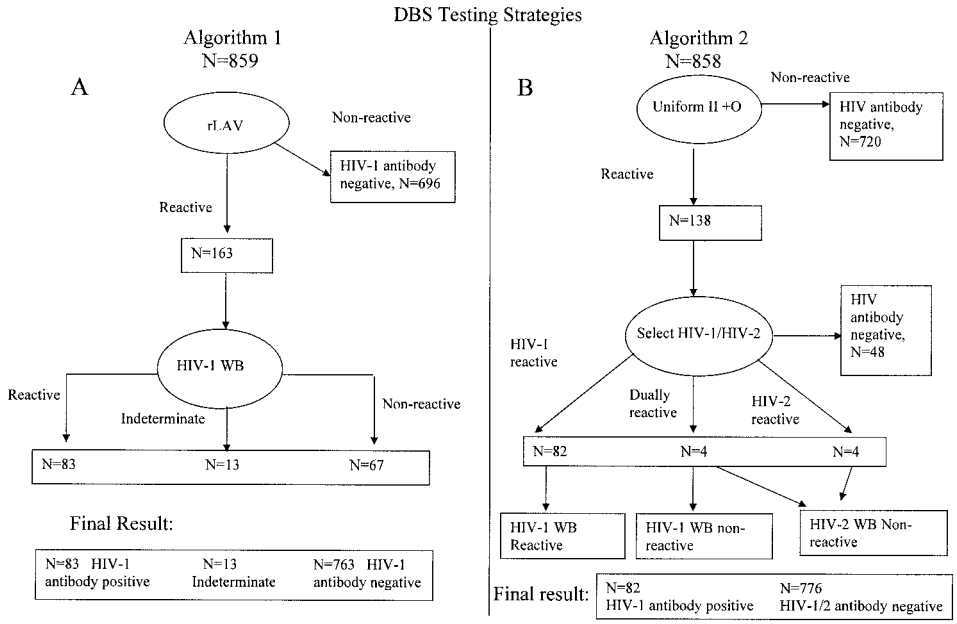

FIG. 3. Comparison of two testing strategies for the determination of HIV antibody status from whole-blood specimens collected on filter paper. N, number.

mens, 4 specimens weakly reactive for HIV-2, and 4 specimens weakly reactive for both HIV-1 and HIV-2. When these 90 specimens were tested by HIV-1 WB and HIV-2 WB, only the 82 HIV-1 antibody-positive specimens were reactive to HIV-1 WB; none were reactive by HIV-2 WB. In this sample set, no additional HIV antibody-positive specimens were identified by the HIV-1/HIV-2 testing of the DBS. All of the HIV-1 WB indeterminate specimens found in the rLAV/WB algorithm (Fig. 3A) were nonreactive when tested by the UNF/SEL testing algorithm for antibodies to HIV-1/HIV-2 (Fig. 3B).

Reagent cost of testing algorithms. The reagent cost of testing 846 specimens by the serial testing algorithm was $1,481 ($1.75 per specimen) and was less than half the cost of the parallel algorithm: $2,894 for the 817 specimens ($3.54 per specimen). These costs reflect all testing done within each algorithm, including the resolution of discordant specimens. Both RT algorithms are significantly less expensive than the $7.76 per specimen cost of the EIA/WB for the DBS specimens. The use of two EIAs (UNF and SEL) for the DBS testing reduced the number of WB tests needed to resolve all specimens to 19 (6 HIV-1, 13 HIV-2) and had an average cost per specimen of $2.34.

DISCUSSION

The World Health Organization (WHO) has encouraged the use of RT in areas where EIA testing is not feasible and where the rapid return of test results can significantly improve counseling and treatment options, such as voluntary counseling and testing (VCT) centers and prenatal clinics (4). In addition to lower costs, a major clinical advantage of RTs is the ability to use them in serial or parallel testing algorithms to screen patient specimens and to retest initial reactive results in a single clinic visit (21). Evaluations of combinations of HIV RTs have yielded 100% sensitivity and specificity; however, these studies have used serum or plasma as the specimen, and most were performed in laboratory settings (6, 8, 22, 23). Our field study in rural Cameroon used whole-blood and plasma specimens from prenatal patients for rapid HIV screening, so that same-day posttest counseling could be done and nevirapine therapy administered to infected women and their infants to prevent mother-to-child HIV transmission. Since this study was conducted, this program has expanded to over 100 facilities in 6 of the 10 provinces of Cameroon (24).

The use of HIV RTs eliminates the indeterminate status that results from the EIA/WB testing algorithm. All of the indeterminate specimens except one were negative by at least two of the RTs and by the UNF and SEL EIAs. Twelve indeterminate specimens had characteristic banding patterns of one to three core antigen-related bands which appear to be nonspecific. In population-based studies, 10 to 15% of the EIA initial reactive specimens may show one or more *gag*-related bands on WB. However, most of these specimens do not represent developing HIV infections (9, 17). The one specimen that was reactive by the RT and had an indeterminate status could have been a recent infection, since WB results displayed some glycoprotein and polymerase antibody reactivity. Thus, the RT results of these 13 WB-indeterminate specimens that were reported to the patient were consistent with the results of the dual EIA testing strategy for the DBS (except for the one RT-positive specimen) and were probably correct. Efforts were made to determine the outcome of the 13 women with indeterminate HIV WB results, but no additional information was available at this time; thus, these data were excluded from the analyses.

The choice of serial versus parallel rapid testing algorithms relates to cost factors as well as performance. The study by Koblavi-Deme et al. demonstrated that the added cost of the parallel algorithm is about 2.5 times as much as the serial

algorithm in reagents alone and was not warranted, since their sensitivity and specificity were not improved (18). A cost analysis of the tests used in this study yielded similar results. In our study, however, two additional HIV antibody-positive specimens would have been identified by the parallel algorithm versus the serial algorithm, albeit with significant increases in reagent costs. The decision on the choice of different RT HIV testing strategies will depend on the performance characteristics of the testing algorithms in a given country or region, the availability of RTs, the purpose of the testing, and the program budgets. For the PMCT program and other VCT in Cameroon, clients with a single negative RT result in the serial RT algorithm are advised about the possible time delay between infection and HIV seropositivity, and they are directed to seek a repeat test in 3 to 6 months. However, blood donations are currently screened using a parallel RT algorithm, which minimizes the risk of HIV infection through contaminated blood products.

Effective methods for providing quality assurance for RT results have not been developed. If DBS specimens were collected at the same time that the RTs were performed, the DBS could be tested later to determine the accuracy of RT results. Tests approved for use with DBS in the United States are not generally available in foreign markets and do not test for antibodies to HIV-2. The third-generation EIAs for the detection of antibodies to HIV now available internationally significantly improved sensitivity by increasing the volume of sample added to the assay. Such volumes are unattainable when eluting DBS, since the amount of serum in a 6-mm punch is approximately 5 μl. However, we did not observe major differences in the ability to detect HIV antibody-positive specimens in our study using the Uniform II plus O assay (only one weakly reactive specimen was not detected). In fact, the signal-to-cutoff ratios (S/CO) were superior to those determined by the rLAV test due to the lower cutoff associated with the UNF assay. Similar observations were noted for the Select HIV-1/2 assay on the limited number of specimens that required additional testing.

The specificities of the UNF and SEL tests were actually superior to that observed with the FDA-licensed rLAV kit, which had a substantial number of repeatedly reactive specimens that were slightly above the cutoff value and had to be resolved by WB. Absorption of moisture by the DBS or exposure to heat are also known to affect DBS quality (15). The initial reactive rate of the DBS specimens tested in this study decreased as personnel became more familiar with DBS collection and storage requirements. With additional modifications, the two EIAs could possibly improve specificity, while yet maintaining the same level of sensitivity. The use of the UNF followed by the SEL test in our study could serve as a model of a dual EIA method for confirming RT results using DBS. Optimal conditions for using existing third-generation EIAs with DBS specimens must be determined and their sensitivity and specificity verified, particularly with recent seroconversion specimens. We are currently evaluating additional EIAs for use with DBS using an elution buffer that would be compatible with the different EIAs and would make a dual EIA testing algorithm for DBS specimens more feasible.

In summary, we have shown the effectiveness of serial and parallel HIV RT algorithms for identifying HIV-infected women in rural clinics in order to institute therapy to reduce perinatal HIV transmission. This program has been greatly expanded in Cameroon, and the efficacy of the intervention strategy is reported elsewhere (24). Additionally, the utility of DBS for confirming HIV RT results and for quality assurance purposes has been demonstrated. Adoption of RT and DBS testing strategies should be considered when expanding HIV testing programs to provide HIV-1 perinatal intervention therapies and to extend seroprevalence studies into global populations.

ACKNOWLEDGMENTS

This work was funded by the Elizabeth Glaser Pediatric AIDS Foundation and supported by the Division of AIDS, STD, and TB Laboratory Research, National Center for Infectious Diseases, and the National Center for HIV, STD and TB Prevention, Centers for Disease Control and Prevention, Atlanta, Ga.

We acknowledge Marcia Kalish, George Alemnji, and Peter Fonjungo, HIV and Retrovirology Branch, and Mark Rayfield, Division of AIDS, STD, and TB Laboratory Research, National Center for Infectious Diseases, at the Centers for Disease Control and Prevention, Atlanta, Ga., for their technical and laboratory support for this work. Andrea Cedfeldt assisted with this evaluation during a Dartmouth Medical School elective. We thank the laboratory staff at CBCHB facilities for their meticulous HIV testing. Thanks to Steve Soroka and Kim Distel for their assistance with editing of the manuscript. We acknowledge the prenatal patients who had the courage to consent to HIV testing to reduce the risk of HIV infection in their children.

Use of trade names is for identification only and does not constitute endorsement by the U.S. Department of Health and Human Services, the Public Health Service, or the Centers for Disease Control and Prevention.

REFERENCES

1. **Anonymous.** 2004. Rapid HIV tests: guidelines for use in HIV testing and counselling services in resource-constrained settings. World Health Organization, Geneva, Switzerland.
2. **Anonymous.** 1997. Blood collection on filter paper for neonatal screening programs; approved standard—third edition, LA4-A3. National Committee for Clinical Laboratory Standards, Wayne, Pa.
3. **Anonymous.** 2001. Guidelines for using HIV testing technologies in surveillance. World Health Organization and Joint United Nations Programme on HIV/AIDS.
4. **Anonymous.** 1998. The importance of simple/rapid assays in HIV testing. Wkly. Epidemiol. Rec. **73:**321–326.
5. **Arai, H., B. Petchclai, K. Khupulsup, T. Kurimura, and K. Takeda.** 1999. Evaluation of a rapid immunochromatographic test for detection of antibodies to human immunodeficiency virus. J. Clin. Microbiol. **37:**367–370.
6. **Behets, F., K. Bishagara, A. Disasi, S. Likin, R. W. Ryder, C. Brown, and T. C. Quinn.** 1992. Diagnosis of HIV infection with instrument-free assays as an alternative to the ELISA and Western blot testing strategy: an evaluation in Central Africa. J. Acquir. Immun. Defic. Syndr. **5:**878–882.
7. **Branson, B.** 2000. Rapid tests for HIV antibody. AIDS Rev. **2:**76–83.
8. **De Cock, K. M., A. Porter, J. Kouadio, M. Maran, E. Gnaore, G. Adjorlolo, M. F. Lafontaine, G. Bretton, G. M. Damet, and K. Odehouri.** 1990. Rapid and specific diagnosis of HIV-1 and HIV-2 infections: an evaluation of testing strategies. AIDS **4:**875–878.
9. **Dock, N. L., H. V. Lamberson, Jr., T. A. O'Brien, D. E. Tribe, S. S. Alexander, and B. J. Poiesz.** 1988. Evaluation of atypical human immunodeficiency virus immunoblot reactivity in blood donors. Transfusion **28:**412–418.
10. **Downing, R. G., R. A. Otten, E. Marum, B. Biryahwaho, M. G. Alwano-Edyegu, S. D. Sempala, C. A. Fridlund, T. J. Dondero, C. Campbell, and M. A. Rayfield.** 1998. Optimizing the delivery of HIV counseling and testing services: the Uganda experience using rapid HIV antibody test algorithms. J. Acquir. Immun. Defic. Syndr. Hum. Retrovirol. **18:**384–388.
11. **Farnham, P. G., R. D. Gorsky, D. R. Holtgrave, W. K. Jones, and M. E. Guinan.** 1996. Counseling and testing for HIV prevention: costs, effects, and cost-effectiveness of more rapid screening tests. Pub. Health Rep. **111:**44–53.
12. **Granade, T. C., S. K. Phillips, C. J. Bell, C. P. Pau, B. Parekh, W. H. Hannon, M. Gwinn, M. A. Redus, G. Schochetman, and J. R. George.** 1992. Factors influencing HIV-1 banding patterns in miniaturized western blot testing of dried blood spot specimens. J. Immunol. Methods **154:**225–233.
13. **Gwinn, M., M. Pappaioanou, J. R. George, W. H. Hannon, S. C. Wasser, M. A. Redus, R. Hoff, G. F. Grady, A. Willoughby, and A. C. Novello.** 1991. Prevalence of HIV infection in childbearing women in the United States. Surveillance using newborn blood samples. JAMA **265:**1704–1708.

14. **Hannon, W. H., D. S. Lewis, W. K. Jones, and M. K. Powell.** 1989. A quality assurance program for human immunodeficiency virus seropositivity screening of dried-blood spot specimens. Infect. Control Hosp. Epidemiol. **10:**8–13.
15. **Hannon, W. H., L. Omar, D. S. Lewis, and S. A. McGee.** 1989. Preparation and characterization of HIV seropositive dried-blood spot materials for quality control and performance evaluation of laboratories, p. 31–36. *In* B. J. Schmidt, A. J. Diament, and N. S. Loghin-Grosso (ed.), Current trends in infant screening. Elsevier, New York, N.Y.
16. **Kassler, W. J., M. G. Alwano-Edyegu, E. Marum, B. Biryahwaho, P. Kataaha, and B. Dillon.** 1998. Rapid HIV testing with same-day results: a field trial in Uganda. Int. J. STD AIDS **9:**134–138.
17. **Kleinman, S., L. Fitzpatrick, K. Secord, and D. Wilke.** 1988. Follow-up testing and notification of anti-HIV Western blot atypical (indeterminant) donors. Transfusion **28:**280–282.
18. **Koblavi-Deme, S., C. Maurice, D. Yavo, T. S. Sibailly, K. N′Guessan, Y. Kamelan-Tano, S. Z. Wiktor, T. H. Roels, T. Chorba, and J. N. Nkengasong.** 2001. Sensitivity and specificity of human immunodeficiency virus rapid serologic assays and testing algorithms in an antenatal clinic in Abidjan, Ivory Coast. J. Clin. Microbiol. **39:**1808–1812.
19. **Ng, K. P., T. L. Saw, A. Baki, J. He, N. Singh, and C. M. Lyles.** 1999. Evaluation of a rapid test for the detection of antibodies to human immunodeficiency virus type 1 and 2. Int. J. STD AIDS **10:**401–404.
20. **Stetler, H. C., T. C. Granade, C. A. Nunez, R. Meza, S. Terrell, L. Amador, and J. R. George.** 1997. Field evaluation of rapid HIV serologic tests for screening and confirming HIV-1 infection in Honduras. AIDS **11:**369–375.
21. **Tamashiro, H., and D. Heymann.** 1993. Reducing the cost of HIV antibody testing. Lancet **342:**87–90.
22. **Urassa, W., S. Nozohoor, S. Jaffer, K. Karama, F. Mhalu, and G. Biberfeld.** 2002. Evaluation of an alternative confirmatory strategy for the diagnosis of HIV infection in Dar Es Salaam, Tanzania, based on simple rapid assays. J. Virol. Methods **100:**115–120.
23. **Urassa, W. K., U. Bredberg-Raden, E. Mbena, K. Palsson, E. Minja, R. A. Lema, K. Pallangyo, F. S. Mhalu, and G. Biberfeld.** 1992. Alternative confirmatory strategies in HIV-1 antibody testing. J. Acquir. Immun. Defic. Syndr. **5:**170–176.
24. **Welty, T., M. Bulterys, P. Tih, G. Ndikintum, G. Nkuoh, J. Nkfusai, J. Kayita, J. Nkengasong, and C. Wilfert.** Integrating prevention of mother-to-child HIV transmission into routine antenatal care: the key to program expansion in rural Cameroon. J. Acquir. Immun. Defic. Syndr., in press.
25. **Wilkinson, D., N. Wilkinson, C. Lombard, D. Martin, A. Smith, K. Floyd, and R. Ballard.** 1997. On-site HIV testing in resource-poor settings: is one rapid test enough? AIDS **11:**377–381.
26. **Zaw, M., R. R. Frerichs, K. Y. Oo, and N. Eskes.** 1999. Local evaluation of a rapid HIV assay for use in developing countries. Trop. Med. Int. Health **4:**216–221.