Rapid Detection and Differentiation of Antibodies to HIV-1 and HIV-2 Using Multivalent Antigens and Magnetic Immunochromatography Testing

Timothy C. Granade,* Shon Workman, Susan K. Wells, Angela N. Holder, S. Michele Owen, and Chou-Pong Pau

National Center for HIV, Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia 30333

Received 20 January 2010/Accepted 11 April 2010

A simplified lateral-flow assay for the detection of antibodies to HIV using magnetic-bead conjugates and multibranched peptides from both HIV-1 and HIV-2 was developed. Magnetic immunochromatography testing (MICT) uses a standard lateral-flow platform that incorporates magnetic-bead conjugates for quantitative measurement of the magnetic field distortion associated with the bound magnetic conjugate (reported as adjusted relative magnetic units [MAR]). The results of the optimized MICT assay were compared to standard enzyme immunoassay (EIA) and Western blotting (WB) results using a blinded 649-member panel of specimens from the United States, Cameroon, and West Africa. The panel was comprised of samples from individuals infected with various HIV-1 subtypes (n = 234) or HIV-2 (n = 65) and HIV-seronegative specimens (n = 350). Additionally, 13 HIV-1 serocconversion panels (total specimens = 85), a worldwide panel containing seven of the major circulating HIV-1 subtypes (n = 18), an HIV-2 panel, an HIV-1/HIV-2 mixed panel, and 100 prospective specimens were tested with completely concordant results. Assay reproducibility (observed MAR) for both intra- and interrun testing was excellent, with coefficients of variation of <12%. MICT can provide a rapid, low-cost method of determining HIV antibody status requiring no subjective interpretations.

The use of rapid HIV antibody-screening assays has permitted the global expansion of HIV testing into rural, nonlaboratory settings and has significantly increased the number of individuals that have been screened. These assays are primarily designed as lateral-flow formats that use colored detection reagents, such as colloidal gold or selenium, conjugated to HIV antigens or to proteins that bind to specific human immunoglobulins, such as protein A or G (10). The tests are rapid, inexpensive, and stable over a broad temperature range and are simple to perform, requiring no additional equipment (1). Although they are generally easy to interpret by visual inspection, there are reports of false-reactive devices, particularly in low-prevalence settings (6). Investigations to determine the sources of these problems have not identified any particular trait other than the subjective interpretation of the results (3). Diagnostic-instrument manufacturers have responded by developing lateral-flow strip readers that use reflectance, fluorescence, and magnetic measurements to provide a more precise and objective result (7, 14). Such devices could also be used to develop quantitative lateral-flow tests for a variety of diagnostic applications.

For rapid HIV testing, lateral-flow tests primarily use HIV-1 subtype B antigens from the immunodominant transmembrane region to capture HIV-specific antibodies. Current commercial assays have been shown to perform well with specimens from individuals infected with other HIV-1 subtypes, even group O (4, 23). However, how these assays perform during early seroconversion with non-subtype B infections has not been assessed, since panels for other subtypes are unavailable. Furthermore, the development of assays for HIV incidence determinations has shown that the immune responses to subtype B antigens are not equivalent across HIV subtypes (21) and that multisubtype antigens are more effective at establishing comparable incidence measurements in international cross-sectional surveys. Thus, detection of antibodies generated to a variety of HIV subtypes might be improved through the use of a broader antigenic mix (chimeric recombinant proteins or synthetic peptides) and/or a more effective antigenic presentation (multibranched peptides), both of which have proven useful in diagnostic assays for HIV and other infectious agents (13, 15, 19, 22).

The purpose of this study was to develop a quantifiable lateral-flow test for the detection and differentiation of antibodies to HIV-1 and HIV-2 using magnetic-bead markers (magnetic immunochromatography test [MICT]). In order to maximize HIV-specific antibody capture, multibranched peptides (MBP) for both HIV-1 and HIV-2 (22) were evaluated for use in a single assay that could detect and differentiate HIV infections. The assay was tested using a 649-member panel of specimens from diverse global locales, 13 HIV-1 serocconversion panels, a panel representing seven of the primary HIV-1 subtypes, an HIV-2 panel, an HIV-1/HIV-2 mixed panel, and 100 prospectively tested specimens. The results were compared to those of standard serological tests, including enzyme immunosassays (EIA), Western immunoblot assays, and a rapid immunosay that is licensed by the U.S. Food and Drug Administration to differentiate HIV-1 and HIV-2 infections. The MICT HIV antibody assay is compatible with available low-
cost equipment, is simple to perform, and produces results in 20 min.

MATERIALS AND METHODS

Specimens. A blinded panel was prepared to evaluate the performance of the optimized MICT assay using specimens collected in CDC epidemiological surveys under CDC-approved protocols (IRB-1896 and IRB-1367), as well as specimens obtained from commercial sources. The panel consisted of 649 serum/plasma specimens from the United States, Cameroon, and West Africa with the following characteristics: 350 nonreactive, 234 HIV-1, and 65 HIV-2. All of the panel members were tested by EIA (Bio-Rad HIV-1/2 plasma specimens from the United States, Cameroon, and West Africa with the assays under CDC-approved protocols (IRB-1896 and IRB-1367), as well as optimized MICT assay using specimens collected in CDC epidemiological sequences.

Establishment of the parameters for the MICT HIV antibody detection assay. The 10 specimens (3 HIV-1, 3 HIV-2, and 4 HIV nonreactive) were selected to be used in experimental protocols to optimize the concentrations of the MICT reagents and to develop an appropriate assay procedure. The specimens were tested in a variety of dilutions from 1:10 through 1:3,200, and the mass of the magnetic-bead conjugate was varied from 10 to 30 μg per test. After the establishment of the optimized assay protocol, an assessment of nonspecific reactivity to the HIV-1 and HIV-2 MBP antigens was performed using a subset of the established HIV-nonreactive specimen panel (n = 265) described above.

Detection of HIV-1 and HIV-2 antibodies in serum and plasma. Serum and plasma specimens were diluted at 1 μl into 100 μl of 10 mM phosphate buffer (pH 7.2) containing 40% chicken serum. Two microliters of protein A magnetic beads (20-μg mass) was added to the tube, and the solution was mixed by vortexing it for 2 s. The specimen was incubated for 2 min, and then 100 μl of the mixture was pipetted into the sample port of the cassette. The reaction lines of the magnetic assays were read at 20 and 40 min using the assay development system (ADS) (MagnaBiosciences, Inc.), which quantitatively measures the magnetic field induced by the captured magnetic particles (14). The amplitude of the detected signal is directly proportional to the amount of magnetic material in the assay reaction zone. The instrument software calculates a relative magnetic unit (MAR) based on the detected magnetic signals, and this value was used to evaluate the performance characteristics of the assay.

Assay reproducibility. Three well-characterized sera—one HIV-1 antibody positive, one HIV-2 antibody positive, and one HIV nonreactive—were selected to evaluate the quantitative capability of the MICT assay to detect and to differentiate HIV-specific antibodies. The reproducibility of the MICT assay was established using multiple runs (n = 10) of the three specimens in triplicate over a 10-day period. Data were analyzed over the period by averaging the quantitative results and determining the coefficients of variation (CV) for both intrarun and interrun data.

RESULTS

Establishment of the parameters for the MICT HIV antibody detection assay. Using the 10 specimens (3 HIV-1, 3 HIV-2, and 4 nonreactive) described above, the optimal dilution of specimens was determined to be between 1:100 and 1:800. Strongly reactive HIV-1 and HIV-2 specimens could be diluted 1:5,000 with almost no decrease in detectable MAR,
and they were still strongly reactive at dilutions of 1:50,000 (data not shown). Although 10 μg of the magnetic-bead conjugate allowed effective identification of HIV antibody-positive specimens, the overall assay sensitivity was improved by increasing the magnetic-bead mass to 20 μg with no impact on assay specificity (data not shown). Quantitative data for the 10 specimens tested using the optimized protocol are shown in Table 1. Both HIV-1 and HIV-2 antibody-positive specimens had MAR values in excess of 200, while the four HIV-nonreactive specimens displayed no reactivity (0 MAR) at either of the HIV peptide antigen lines. Cross-reactivity of the HIV-1 and HIV-2 antibodies to the MBP in these specimens was not noted after 20 min of incubation.

Non-specific reactivity of the HIV antibody-nonreactive specimens (n = 265) to both the HIV-1 and HIV-2 MBP was low; all of the specimens had MAR values of <10 (Fig. 2). Based on these data, a cutoff of 15 MAR was established that would clearly distinguish both HIV-1 and HIV-2 antibody-positive samples from HIV antibody-nonreactive specimens. This cutoff was determined using the average MAR of nonreactive specimens on the HIV-1 MBP (1.9 MAR) plus 3 standard deviations (3.5 MAR) and was used throughout the rest of the analysis.

**TABLE 1. MICT detection of antibodies to HIV-1 and HIV-2 after 20 and 40 min of incubation.**

| Specimen no. | HIV reactivity | MAR<sup>a</sup> | 20 min | 40 min |
|--------------|---------------|-----------------|--------|--------|
|              |               | HIV-1 | HIV-2 | HIV-1 | HIV-2 |
| 1            | HIV-1         | 535.8 | 0.0   | 1,134.2 | 0.0 |
| 2            | HIV-1         | 750.6 | 0.0   | 1,241.9 | 0.0 |
| 3            | HIV-2         | 1,099.5 | 0.0 | 1,821.6 | 0.0 |
| 4            | HIV-2         | 0.0   | 461.6 | 0.0   | 1,054.8 |
| 5            | HIV-2         | 0.0   | 200.6 | 0.0   | 412.0 |
| 6            | HIV-2         | 0.0   | 766.6 | 0.0   | 1,531.5 |
| 7            | Nonreactive   | 0.0   | 0.0   | 0.0   | 0.0 |
| 8            | Nonreactive   | 0.0   | 0.0   | 0.0   | 0.0 |
| 9            | Nonreactive   | 0.0   | 0.0   | 0.0   | 0.0 |
| 10           | Nonreactive   | 0.0   | 0.0   | 0.0   | 0.0 |

<sup>a</sup> Data shown are MAR values to the HIV-1 and HIV-2 MBP antigens. Specimens were diluted 1:100 in assay running buffer. Assays were run using 20 μg/ml of magnetic-bead conjugate.

**FIG. 2.** MAR values for HIV antibody-nonreactive specimens (n = 265) on the MBP antigen lines for HIV-1 and HIV-2. Over 65% of the specimens had no detectable MAR against either MBP antigen, and no specimen produced MAR values of >10. The MAR values were obtained after 20 min of incubation.

**TABLE 2. Detection of antibodies to HIV-1 and HIV-2 by MICT compared to detection of HIV-1 and HIV-2 antibodies by the EIA/WB reference standard.**

| EIA/WB result | MICT result<sup>a</sup> |
|---------------|-------------------------|
| HIV-1 reactive | HIV-2 reactive | HIV nonreactive |
| HIV-1 positive | 234 | 0 | 0 |
| HIV-2 positive | 0 | 65 | 0 |
| HIV negative | 0 | 0 | 350 |

<sup>a</sup>MICT results were determined at 20 min. Sensitivity and specificity were 100%.

Detection of HIV antibodies by MICT. The performance characteristics of the optimized MICT assay were evaluated using the specimen panel (n = 649) described in Materials and Methods. The data were compared to results from the standard EIA/WB testing (reference standard) (Table 2). The MICT rapid test had excellent sensitivity and specificity and correctly identified the 234 HIV-1 antibody-positive specimens, the 65 HIV-2-antibody positive specimens, and the 350 nonreactive specimens. The quantitative MAR values of the MICT assay clearly differentiated HIV-1 from HIV-2 antibody-positive specimens based on the MAR value. In fact, cross-reactivity between the two MBPs was very low, with MAR readings of antibodies to one of the HIV types normally below the cutoff of 15 MAR on the MBP to the other type (data not shown). All 10 members of the HIV-2 panel (PRF201) were also reactive to only the HIV-2 MBP and did not have any cross-reactivity to the HIV-1 MBP. The mixed panel of HIV-1 and HIV-2 specimens (PRZ 201; SeraCare, Inc.; n = 15) was also run to further validate the ability of the assay to detect and to differentiate HIV-1 and HIV-2 antibodies. The HIV-1 (n = 7) and HIV-2 (n = 6) antibody-positive specimens were detected with MAR values of >150 for HIV-1 and >300 for HIV-2. The antibody-negative and WB-indeterminate specimens in the panel had MAR values of <15 to both of the HIV MBP antigens (data not shown). The MICT assay also detected all of the members of the worldwide panel that included seven of the major group M subtypes, as well as some of the circulating recombinant forms (Table 3). The MAR values observed on the panel ranged from 157.0 for one of the subtype A specimens to over 1,800 MAR for one of the AG recombinant forms, and cross-reactivity to the HIV-2 MBP was not observed.

Ability to detect HIV-1 seroconversion. The ability to detect HIV-1 seroconversion was assessed using the 13 commercial panels. The MICT data were compared to data from first-, second-, and third-generation EIAs and Western blot results derived from the panel inserts that were provided by the panel supplier (Table 4). Of the 85 specimens in the collection, 43 were antibody positive by the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA, a third-generation HIV test that both captures and detects HIV antibodies using HIV-specific antibodies. Only 32 of these specimens were denoted HIV antibody-positive by WB analysis. The MICT assay performed better than WB, detecting 34 of the specimens, which was fewer than were detected by the third-generation EIA but was consistent with or better than the first-generation (Abbott HIV-1) and second-
TABLE 3. MICT detection of antibodies in the worldwide panel containing specimens from individuals infected with HIV-1 subtypes and circulating recombinant forms

| Specimen no. | HIV-1 subtypes | MAR at 20 min | CTL* | HIV-1 | HIV-2 |
|--------------|----------------|---------------|------|-------|-------|
| 1            | C              | 2,003.0       | 1,244.0 | 0.0   |
| 2            | C              | 1,995.4       | 1,821.4 | 0.0   |
| 3            | CRF01/AE       | 3,260.0       | 1,121.7 | 0.0   |
| 4            | B              | 2,911.8       | 398.2  | 0.0   |
| 5            | B              | 3,148.6       | 908.1  | 0.0   |
| 6            | G              | 2,654.9       | 814.1  | 0.0   |
| 7            | A              | 2,800.3       | 157.0  | 0.0   |
| 8            | G              | 2,579.6       | 667.0  | 0.0   |
| 9            | CRF02/AG       | 2,424.6       | 593.9  | 0.0   |
| 10           | F              | 2,672.3       | 403.9  | 0.6   |
| 11           | H              | 1,645.6       | 1,723.9 | 0.0   |
| 12           | G              | 2,685.9       | 277.3  | 0.0   |
| 13           | CRF02/AG       | 1,834.3       | 1,818.7 | 0.0   |
| 14           | D              | 3,275.4       | 712.7  | 0.0   |
| 15           | A              | 2,095.7       | 1,091.1 | 0.0   |
| 16           | A              | 1,998.6       | 1,699.1 | 0.0   |
| 17           | C              | 2,629.6       | 793.8  | 0.0   |
| 18           | CRF01/AE       | 2,730.0       | 1,155.2 | 0.0   |

* CTL, protein A control.

Prospective evaluation of the MICT HIV antibody detection assay. Samples arriving for routine screening in the HIV reference laboratory with sufficient volume remaining were further tested by the MICT assay, and the results were compared to those of the current testing algorithm of EIA/WB (Bio-Rad HIV-1/2 + O EIA and Bio-Rad HIV-1 WB). Of the 100 specimens that arrived in the laboratory, 18 were HIV-1 antibody positive, three were HIV-2 antibody positive, 60 were nonreactive, and 19 were EIA reactive-WB indeterminate by the standard algorithm. Since the ultimate status of indeterminate specimens was not known, MICT testing was not performed on those samples. The MICT results for the remaining 81 specimens were fully concordant with the standard EIA/WB testing results.

The reproducibility of the MICT assay was established using multiple runs (n = 10) of the three specimens in triplicate over a 10-day period (Table 5). MAR readings for both of the HIV-reactive specimens were high (HIV-1 average = 717; HIV-2 average = 838), while the nonreactive sera had no detectable MAR against either of the HIV branched-peptide antigens. CV for all of the measurable MAR for HIV-1 antibodies were less than 8.2% and were independent of the incubation time (20 versus 40 min). CV for detection of the antibodies to HIV-2 were slightly higher (~12%) and were also time independent.

DISCUSSION

The use of rapid HIV tests has now become routine in many areas of the world due to their simplicity, low cost, and excellent performance characteristics (8, 24). In the United States, six rapid HIV assays are now approved by the U.S. Food and Drug Administration, and four of these have been categorized as "waived" testing under CLIA 1988, where the qualifications of the testers are minimal. These traits have led to expansion of testing into a variety of nonlaboratory venues worldwide and have allowed extended outreach to at-risk populations (6, 17, 20, 25). Although some studies have shown that nonlaboratorians can effectively perform these waived tests (12), others have detected problems with HIV rapid testing, as well as with simple tests for other infectious diseases or clinical syndromes (9). One of the primary problems has been the subjective nature of the test result, which must be individually interpreted by the test performer. This particular characteristic has led to interpretive problems that could not be readily resolved de-
spite investigation (3). The MICT assay presented here alleviates these concerns by having a quantifiable, objective result that can be used for assay interpretation. The assay retains the advantages of existing rapid HIV antibody detection assays but uses an inexpensive, stand-alone magnetic reader to detect and differentiate the presence of antibodies to HIV-1 and HIV-2 in a single device.

MBP have been used for a number of years to improve the sensitivity and specificity of diagnostic assays. The multivalent and multiepitope antigens used in the MICT assay described here permitted the detection of all of the HIV antibody-positive specimens in the test panel, which consisted of samples collected in diverse global locales and likely included members from individuals infected with various HIV-1 group M subtypes. Members of the global panel included specimens from individuals infected with subtypes A to G and some circulating recombinant forms. All were detected by the MICT assay, indicating that these antigens provided good coverage for antibodies elicited by the major group M subtypes of HIV-1. Similarly, the antigens used for the detection of HIV-2 specimens also performed well on the smaller HIV-2 antibody-positive specimens included in the panel.

One unique feature of this evaluation was the low cross-reactivity that was observed between the HIV-1 and HIV-2 MBP antigens in HIV antibody-positive specimens. Serological differentiation of HIV-1 and HIV-2 infections was initially difficult in areas where both viruses circulate due to the cross-reactivity of the antibodies elicited by the two viruses (5). However, some of the peptide-based rapid assays have been formatted to differentiate HIV infections, and some of these have been shown to be effective in this regard (2, 18). The MBPs used in the MICT assay provided excellent ability to both detect and differentiate HIV-1 and HIV-2 antibodies, with only a few cross-reactive specimens observed (data not shown).

The quantitative nature of the MICT assay could improve sensitivity and specificity through the quantitative determination of the magnetic field associated with true binding events. The MAR values determined for both MBP antigens were essentially zero for HIV antibody-negative samples, with only a few cross-reactive specimens observed (data not shown).

The findings and conclusions in this paper are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

ACKNOWLEDGMENT

The findings and conclusions in this paper are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

REFERENCES

1. Anonymous. 2002. HIV simple/rapid assays: operational characteristics 12. World Health Organization, Geneva, Switzerland.
2. Brattegaard, K., J. Koudou, M. L. Adom, R. Doorly, J. R. George, and K. M. De Cock. 1993. Rapid and simple screening and supplemental testing method for HIV-1 and HIV-2 infections in west Africa. AIDS 7:583–885.
3. Centers for Disease Control and Prevention. 2008. False-positive oral fluid rapid HIV tests—New York City, 2005–2008. MMWR Morbid. Mortal. Weekly Rep. 57:660–666.
4. Constantine, N. T., L. Zekeng, A. K. Sangare, L. Gurtler, R. Saville, H. Anhary, and C. Wild. 1997. Diagnostic challenges for rapid human immunodeficiency virus assays. Performance using HIV-1 group O, HIV-1 group M, and HIV-2 samples. J. Hum. Virol. 1:45–51.
5. De Cock, K., A. Porlier, J. Koudadio, et al. 1991. Cross-reactivity on Western blots in HIV-1 and HIV-2 infections. AIDS 5:859–863.
6. Delaney, K. P., B. M. Branson, A. Uniyal, P. R. Kerndt, P. A. Keenan, K. Jafa, A. D. Gardner, D. J. Jamieson, and M. Bulteys. 2006. Performance of an oral fluid rapid HIV-1/2 test: experience from four CDC studies. AIDS 20:1655–1660.
7. Faulstich, K., R. Gruier, M. Eberhard, and K. Haberstroh. 2007. Developing rapid mobile POC systems. Part 1. Devices and applications for lateral-flow immunodiagnostic tests. IVD Technol. 13:41–53.
8. Franco-Paredes, C., L. Tellez, and C. del Río. 2006. Rapid HIV testing: a review of the literature and implications for the clinician. Curr. HIV/AIDS Rep. 3:169–175.
9. Granade, T. C., B. S. Parekh, S. K. Phillips, and J. S. McDougal. 2004. Performance of the OraQuick and Hema-Slide rapid HIV antibody detection assays by non-laboratorians. J. Clin. Virol. 30:229–232.
10. Granade, T. C., B. S. Parekh, P. M. Tih, T. Welty, E. Welty, M. Bulteys, G. Nlikintum, G. Nkoub, and S. Tancho. 2005. Evaluation of rapid prenatal human immunodeficiency virus testing in rural Cameroon. Clin. Diagn. Lab. Immunol. 12:855–860.
11. Gürtler, L., A. Mulhibacher, U. Michl, H. Hofmann, G. G. Paggi, V. Bossi, R. Thurstenssson, R. Vilaesa, E. Meiras, J. M. Hernandez, W. Melchior, F. Donie, and B. Weber. 1998. Reduction of the diagnostic window with a new combined p24 antigen and human immunodeficiency virus antibody assay. J. Virol. Methods 75:27–38.
12. Kanal, K., T. L. Chou, L. Sovann, Y. Morikawa, Y. Mukoyama, and K. Kakimoto. 2005. Evaluation of the proficiency of trained non-laboratory health staff and laboratory technicians using a rapid and simple HIV antibody test. AIDS Res. Ther. 2:5.
13. Kim, P., and C. P. Pau. 2001. Comparing tandem repeats and multiple antigenic peptides as the antigens to detect antibodies by enzyme immunoassay. J. Immunol. Methods 257:51–54.
14. Laborde, R., and B. O’Farrell. 2002. Paramagnetic-particle detection in lateral flow assays. IVD Technol. 13:36–40.
15. Leahy, D., J. Kink, R. Byrne, D. Shah, B. Preisel-Simmons, S. Laska, J. Dienstag, H. Thomas, P. Karayiannis, and J. Brown. 1992. Improved serologic detection of hepatitis C virus with a paramagnetic microparticle assay using multiple antigenic sequences. Transfusion 32:546–553.
16. Ly, T. D., S. Lapereche, and A. M. Courouce. 2001. Early detection of human immunodeficiency virus infection using third- and fourth-generation screening assays. Eur. J. Clin. Microbiol. Infect. Dis. 20:104–110.
17. Morin, S. F., G. Khumalo-Sakutukwa, E. D. Charlebois, J. Routh, K. Fritz, T. Lane, T. Vaki, A. Fiamma, and T. J. Coates. 2006. Removing barriers to knowing HIV status: same-day mobile HIV testing in Zimbabwe. J. Acquir. Immune Defic. Syndr. 41:218–224.

18. O'Conell, R. J., and S. A. Peel. 2007. Multispot HIV-1/HIV-2 Rapid Test: advantages over other rapid HIV tests. Exp. Rev. Mol. Diagn. 7:499–505.

19. Oladepo, D. K., P. E. Klapper, and H. S. Marsden. 2000. Peptide based enzyme-linked immunoassays for detection of anti-HSV-2 IgG in human sera. J. Virol. Methods 87:63–70.

20. Pai, N. P., J. P. Tulsky, D. Cohan, J. M. Colford, Jr., and A. L. Reingold. 2007. Rapid point-of-care HIV testing in pregnant women: a systematic review and meta-analysis. Trop. Med. Int. Health 12:162–173.

21. Parekh, B. S., and J. S. McDougal. 2005. Application of laboratory methods for estimation of HIV-1 incidence. Indian J. Med. Res. 121:510–518.

22. Pau, C. P., W. Luo, and J. S. McDougal. 2007. Chimeric multiple antigenic peptides for simultaneous detection of specific antibodies to HIV-1 groups M, N, O, and HIV-2. J. Immunol. Methods 318:59–64.

23. Phillips, S., T. C. Granade, C. P. Pau, D. Candal, D. J. Hu, and B. S. Parekh. 2000. Diagnosis of human immunodeficiency virus type 1 infection with different subtypes using rapid tests. Clin. Diagn. Lab. Immunol. 7:698–699.

24. Plate, D. K., and Rapid HIV Test Evaluation Working Group. 2007. Evaluation and implementation of rapid HIV tests: the experience in 11 African countries. AIDS Res. Hum. Retrovir. 23:1491–1498.

25. Steen, T. W., K. Seipone, L. Gomez Fde, M. G. Anderson, M. Kejelepula, K. Keapoletswe, and H. J. Moffat. 2007. Two and a half years of routine HIV testing in Botswana. J. Acquir. Immune Defic. Syndr. 44:484–488.

26. Weber, B., E. H. Fall, A. Berger, and H. W. Duerr. 1998. Reduction of diagnostic window by new fourth-generation human immunodeficiency virus screening assays. J. Clin. Microbiol. 36:2235–2239.

27. Workman, S., S. K. Wells, C. P. Pau, S. M. Owen, X. F. Dong, R. LaBorde, and T. C. Granade. 2009. Rapid detection of HIV-1 p24 antigen using magnetic immuno-chromatography (MICT). J. Virol. Methods 160:14–21.