JOURNAL OF CLINICAL MICROBIOLOGY, July 2011, p. 2590–2593 Vol. 49, No. 7
0095-1137/11/$12.00 doi:10.1128/JCM.02402-10

RNA versus DNA (NucliSENS EasyQ HIV-1 v1.2 versus Amplicor HIV-1 DNA Test v1.5) for Early Diagnosis of HIV-1 Infection in Infants in Senegal[▽]

K. Kébé, O. Ndiaye, H. Diop Ndiaye, P. Mbakob Mengue, P. M. M. Guindo, S. Diallo, N. Léye, S. B. Gueye, A. Gaye Diallo, C. Touré Kane,* and S. Mboup

Laboratoire de Bactériologie Virologie, Hôpital Aristide le Dantec, Université Cheikh Anta Diop, Dakar, Senegal

Received 26 November 2010/Returned for modification 15 February 2011/Accepted 1 April 2011

The objective of this study was to compare the performance of the NucliSENS EasyQ HIV-1 v1.2 platform (bioMérieux, France) to the Amplicor HIV-1 DNA test v1.5 (Roche Molecular Systems, Switzerland) in detecting HIV-1 infection in infants using venipuncture-derived whole blood in tubes and dried blood spots. A total of 149 dried blood spots and 43 EDTA-anticoagulated peripheral blood samples were collected throughout Dakar and other areas in Senegal from infants and children aged 3 weeks to 24 months who were born to HIV-1-infected mothers. Samples were tested using the NucliSENS and Amplicor technologies. The NucliSENS and Amplicor results were 100% concordant using either EDTA-anticoagulated peripheral blood or dried blood spots. Compared to Amplicor, the sensitivity and specificity of the NucliSENS test were 100%. The NucliSENS EasyQ HIV-1 RNA assay performed as well as the Amplicor HIV-1 DNA test in detecting HIV-1 infection in infants. In addition, this platform can give an indication of the viral load baseline. The NucliSENS EasyQ platform is a good alternative for early infant diagnosis of HIV-1 infection.

In 2009, the number of sub-Saharan children infected by the human immunodeficiency virus (HIV) was estimated to be 2.3 million (30). Mother-to-child transmission is the most significant cause of HIV-1 infection and mortality in children under 15 years of age, especially during the first 24 months of life (25), creating an urgent need for early management of these children. Recent studies describing the management and care of newborns infected with HIV have shown that antiretroviral therapy should be implemented during the first 3 months of life (31). This can only be achieved by neonatal screening for HIV infection. However, accessibility can be a limiting factor because commonly available serological tests cannot be used with newborns due to the persistence of maternal antibodies until the age of 18 months (18). The diagnosis is therefore based on other tests, such as viral culture (12), P24 antigenemia, and detection of virus genetic material (26). Viral culture requires specific biosafety equipment, is highly time-consuming, and has low sensitivity (3, 6, 18). P24 antigen detection has lower sensitivity than molecular biology techniques (14), and the P24 antigen may pass through the placental barrier and be detected in noninfected newborns (20). These constraints led to the use of molecular biology techniques to diagnose HIV infection in newborns. The amplification of proviral DNA remains the reference technique for the neonatal diagnosis of HIV infection (7). This test can be performed within 48 h, whereas viral culture can take 14 to 28 days. The Amplicor HIV-1 DNA Test v1.5, developed by Roche Laboratories, is recommended for the identification of HIV-1 infection in children under 18 months of age. It is the only technique that has been validated in several studies in Africa and is widely used in North America, Europe, and Asia (1, 2, 4, 23, 32).

The use of dried blood spots (DBS) has been proposed since the 1990s to make neonatal diagnosis accessible to rural zones that are often far away from reference centers (1, 2, 4, 27). DBS are easy to obtain and are a convenient method for collecting and transporting blood samples because they have less restrictive transport requirements than whole blood in tubes. Several techniques for detecting proviral DNA use DBS for early infant HIV-1 diagnosis (11, 13, 15, 23, 29). Some have been developed in-house, while others are commercial, such as the validated Amplicor technique, which has sensitivity on DBS of 98.2 to 100% and a specificity of 98.6 to 100% (15, 16, 23). The high sensitivity of plasma HIV RNA quantification techniques now allows their substitution for DNA PCR tests (10, 22, 28). These techniques could be used also for early infant diagnosis (EID) because most infected children in these areas do not have the opportunity to be diagnosed before the age of 18 months.

As part of the Prevention of Mother to Child Transmission (PMTCT) initiative in Senegal, EID was first performed in a pilot phase in 2000 using a qualitative in-house PCR method. In 2007, the Amplicor test was introduced and also used DBS.

The widespread availability of RNA techniques in Senegal, as in other countries, and the validation of RNA quantitation on DBS (8, 9, 19) offered an opportunity to evaluate its use in EID.

The objective of the present study was to evaluate the performance of viral RNA detection with the NucliSENS system versus the reference technology, Amplicor, which detects proviral DNA and improves EID in Senegal. Early HIV testing can help HIV-infected infants access treatment, provide reassurance for families of uninfected infants, and help PMTCT programs monitor their effectiveness.

* Corresponding author. Mailing address: Laboratoire de Bactériologie Virologie, Hôpital Aristide le Dantec, 30 Avenue Pasteur, BP 7325, Dakar, Senegal. Phone: 221 33 8225919. Fax: 221 33 821 38 25. E-mail: ctourekane@yahoo.co.uk.

▽ Published ahead of print on 4 May 2011.

MATERIALS AND METHODS

Patients. In total, 192 blood samples were collected from 101 children born to HIV-1-infected mothers. Of these, 99 children were in the PMTCT program and aged from 3 weeks to 18 months and 2 were 24-month-old infants with repeated indeterminate HIV serology. Two types of samples were collected: whole blood in EDTA tubes and DBS. Venous blood samples ($n = 43$) were collected at Albert Royer, the children's reference hospital, and DBS ($n = 149$) were collected at PMTCT sites in the suburbs of Dakar and three other cities in Senegal (Mbour, Joal, and Thiès).

Venipuncture blood sample processing. Blood samples were collected in EDTA tubes by venous puncture and transported daily to the reference laboratory within 6 h of collection. They were then centrifuged at 2,500 rpm for 10 min, and the buffy coat was separated from the plasma and stored at −20°C, while plasma samples were stored at −80°C.

Preparation of DBS. DBS were prepared by spotting several drops of blood collected from the heel or big toe of the child onto Whatman 903 filter paper. The preprinted circles were completely filled. After air-drying overnight at room temperature (22 to 37°C), the Whatman filter papers were stored in plastic zipper bags with a silica desiccant and a humidity indicator. The bags were sent in an envelope to the reference laboratory, where they were kept at room temperature (22 to 25°C) until tested. The median storage duration was 37 days.

DNA Amplicor testing. The Roche Amplicor HIV-1 DNA Test v1.5 is a qualitative *in vitro* test for the detection of HIV-1 DNA in whole blood (17). The test utilizes amplification of target DNA by the PCR and nucleic acid hybridization for the detection of HIV-1 DNA in human whole blood. Both types of specimens (buffy coat and DBS) can be used in this platform. The Amplicor HIV-1 DNA Test v1.5 is based on four major processes: (i) sample preparation, (ii) PCR amplification of target DNA using HIV-1-specific complementary primers, (iii) hybridization of the amplified products to target-specific oligonucleotide probes, and (iv) colorimetric detection of the probe-bound amplified products. For both specimen types, the blood is washed (the DBS is cut out before handling) to collect the leukocytes by centrifugation for lysis with detergent and proteinase K at 60°C for 30 min and at 100°C for 30 min. Then, a target 155-bp region of the *gag* gene is amplified. The Amplicor HIV-1 DNA Test v1.5 permits simultaneous PCR amplification of HIV-1 target and internal control (IC) DNA. The Master Mix reagent contains a biotinylated primer pair specific for both HIV-1 and IC DNA. The detection of amplified DNA is performed using target-specific oligonucleotide probes that permit the independent identification of the HIV-1 IC amplicon, which is performed at the user's option.

For a valid run, sample results were interpreted as follows. If the HIV-1 A_{450} result was <0.2 and the IC A_{450} result was ≥0.2, HIV-1 DNA was not detected, and the sample was presumed negative for HIV-1 DNA. If the HIV-1 A_{450} result was <0.2 and the IC A_{450} result was <0.2, the sample was considered inhibitory. HIV-1 DNA, if present, would not be detectable. Another aliquot of the original sample was processed, and the test was repeated. If the HIV-1 A_{450} result was ≥0.8 and the IC A_{450} result had any value, HIV-1 DNA was detected, and the sample was positive for the presence of HIV-1 DNA. Finally, if the HIV-1 A_{450} result was between 0.2 and 0.8 with any IC A_{450} value, the results seemed inconclusive for HIV-1 DNA. Repeat testing in duplicate was performed regardless of the IC result.

NucliSENS EasyQ HIV-1. This technique also targets the *gag* sequence and allows the quantification of HIV-1 and some HIV-2 subtypes in a range of 25 to 1,000,000 RNA copies in 1 ml of plasma (21). As with Amplicor, both types of specimen collection can be used with the NucliSENS platform. HIV-1 RNA extraction was performed with NucliSENS miniMAG using Boom technology with magnetic silica.

For DBS, one blood spot was completely excised using sterilized scissors that were swabbed with 0.1% bleach solution and dried between samples. The spot was placed in 2 ml of lysis buffer containing guanidine thiocyanate and Triton X-100 (bioMérieux, Lyon, France) and gently rocked for 30 min at room temperature. The filter paper was then removed from the tube, and the solution containing the extracted nucleic acids was further processed. Under high-salt conditions, nucleic acids bind to the silica particles. These silica particles act as a solid phase, and non-nucleic acid components are removed by several washing steps performed in NucliSENS miniMAG. Finally, the nucleic acids are eluted from the solid phase.

In the case of EDTA-anticoagulated peripheral blood, 50 μl of plasma was processed according to the manufacturer's instructions in 2 ml of lysis buffer. The extraction was performed manually using the NucliSENS isolation kit according to the manufacturer's instructions. A synthetic calibrator of known HIV-1 concentration was added as an internal standard. The RNA extraction was followed by a nucleic acid sequence-based amplification and real-time detection with the NucliSENS EasyQ analyzer (bioMérieux) using 5 μl of the eluate according to the manufacturer's instructions.

Results were validated for each sample according to the amplification of the internal control.

Statistical analysis. To evaluate the performance of the NucliSENS EasyQ platform, the Amplicor test was used as a reference. Statistical analyses were performed using EPI INFO version 6 and Microsoft Excel 2003 software. The confidence intervals were 95% (95% CIs) and were calculated by using a normal distribution. The diagnostic accuracy of the NucliSENS EasyQ assay was assessed using the sensitivity (the number of true positives/[true positives + false negatives]), specificity (the number of true negatives/[true negatives + false positives]), positive predictive value (PPV) (the number of true positives/[true positives + false positives]), and negative predictive value (NPV) (the number of true negatives/[false negatives + true negatives]). StatCalc was used to determine the kappa coefficient of agreement, which is an index that compares the agreement against that which might be expected by chance. Kappa can be thought of as the chance-corrected proportional agreement, and possible values range from +1 (perfect agreement) to 0 (no agreement above that expected by chance) to −1 (complete disagreement).

TABLE 1. Sensitivity and specificity of the NucliSENS test on venipuncture derived blood and DBS

Amplicor result	NucliSENS result (no. of samples) using:					
	Venipuncture-derived blood			DBS		
	Positive	Negative	Total	Positive	Negative	Total
Positive	10	0	10	19	0	19
Negative	0	33	33	0	130	130
Total	10	33	43	19	130	149

RESULTS

Comparison using venipuncture-derived whole blood. Among the 43 venipuncture-derived whole-blood samples, HIV-1 RNA was detected in the plasma of 10 positive patients who had also tested positive using buffy coat with the Amplicor test. Therefore, NucliSENS showed 100% sensitivity and specificity (Table 1). The PPV and NPV were also 100%. A perfect concordance was found between the two tests on venipuncture derived blood with a kappa coefficient of 1 ($P < 0.001$, Table 1).

Comparison between NucliSENS and Amplicor showed a sensitivity of 100% (95% CI = 65.5 to 100%), a specificity of 100% (95% CI = 87 to 100%), a PPV of 100% (95% CI = 65.5 to 100%), and an NPV of 100% (95% CI = 87 to 100%).

Comparison to DBS. HIV-1 RNA was detected by NucliSENS in all 19 DBS that also tested positive by Amplicor. No specimen tested positive by Amplicor but negative by NucliSENS. The statistical analysis gave a kappa coefficient of 1 ($P < 0.001$), showing perfect concordance between the two tests with DBS (Table 1). The performance comparison between NucliSENS and Amplicor showed a sensitivity of 100% (95% CI = 79.1 to 100%), a specificity of 100% (95% CI = 96.4 to 100%), a PPV of 100% (95% CI = 79.1 to 100%), and an NPV of 100% (95% CI = 96.4 to 100%).

Viral loads and optical density. Tables 2 and 3 summarize the distribution of HIV-1 RNA viral loads among infected infants in DBS and venipuncture-derived whole blood, respectively. The median interquartile range (IQR) viral load among positive samples was high: 410,000 IU/ml (1,900 to 59,000,000 IU/ml). The median IQR optical density was 2.951 (1.073 to

TABLE 2. Ranges of positive detection in infants by Amplicor and NucliSENS using positive DBS

Sample	Subject age (mo)	OD[a] (Amplicor)	Viral load (IU/ml) (NucliSENS)
3312-hald	3	2.881	14,000
3238-hald	7	3.062	32,000
3225-hald	6	2.664	36,000
2842-haldb	7	2.774	97,000
3190-hald	24	3.129	210,000
3427-hald	8	2.737	556,800
3094-hald	6	2.438	817,800
2947-halda	12	3.123	120,000
3016-hald	9	2.979	130,000
2844-hald	12	2.996	160,000
2830-hald	2	3.011	160,000
2947-haldb	15	3.027	170,000
2977-hald	2	3.008	1,900
2842-halda	1.5	2.764	22,000
3107-hald	12	1.249	38,000
3112-hald	3	2.959	410,000
3138-hald	24	2.91	480,000
18DK0002	4	3.047	520,000
2943-hald	5	2.948	520,000

[a] OD, optical density.

TABLE 3. Ranges of positive detection in infants by Amplicor and NucliSENS using positive venipuncture-derived blood

Sample	Subject age (mo)	DNA-derived whole blood OD[a] (Amplicor)	Plasma RNA viral load (IU/ml) (NucliSENS)
2853-hald	5	2.986	650,000
3064-hald	9	3.075	3,100,000
3091-hald	1.5	1.073	7,400,000
3176-hald	4	2.935	17,000,000
3177-hald	7	2.901	470,000
3178-hald	5	2.951	1,100,000
3179-hald	3	3.075	59,000,000
3020-hald	16	2.609	2,400,000
653hpd	5	3.129	13,000,000
A3-348	5	2.835	7,600,000

[a] OD, optical density.

3.129). A level of ≥5,000 IU/ml is considered positive, while tests with results lower than that value should be repeated based on a new extraction of the sample. In the present study, only one sample showed a viral load of <5,000 IU/ml, but its optical density using Amplicor was 3.008, allowing us to consider this sample HIV-1 positive.

DISCUSSION

The comparison of results from venipuncture samples in EDTA tubes shows high concordance between the two techniques, with 100% sensitivity and specificity for the NucliSENS EasyQ assay. These findings are supported by Young et al. (32, who published identical results in 2000 in their study of 2-month-old children in Thailand whose venipunctures were tested by PCR analyses of RNA and DNA.

The sensitivity and specificity of NucliSENS were 100% compared to Amplicor with DBS samples. The Amplicor technology was validated with HIV-1 serology at 18 months of life as the reference, and a similar study is now needed to determine the sensitivity and specificity of the NucliSENS technology.

NucliSENS is an isothermal RNA amplification technique with real-time detection, combining manual extraction with simultaneous amplification and detection by fluorescence quantification. The major advantage of NucliSENS is that it provides information not only on infection but also on the level of viral replication. The NucliSENS technique is also simpler, easier to implement, and quicker (4 h), particularly the extraction, than other commercialized plasma RNA quantification kits, notably Roche's Amplicor kit (28). The DNA-based Amplicor technique is the reference for early diagnosis of HIV-1 infection in children, and its performance with venipuncture and filter paper samples has been validated in many studies in northern and southern countries (24). All our results were validated by the proviral DNA Amplicor technique and therefore confirm the diagnostic performance of the NucliSENS technique.

In Thailand, where the recombinant HIV-1 subtype (CRF01_AE) is predominant (9), research has shown that Amplicor sensitivity with buffy coat samples increases from 40% at the time of birth to 100% at the age of 2 months, and its specificity is 100% regardless of age. Studies performed in the United States compared the use of proviral DNA and plasma RNA for neonatal diagnosis. The sensitivity of all of these tests varies according to age. Among neonates under the age of 3 weeks, sensitivities of 27% for DNA PCR, 64% for quantitative RNA PCR, and 55% for qualitative tests were found. These same tests have sensitivities of 96.2% in neonates aged between 4 and 6 weeks and 100% at >7 weeks. The DNA PCR specificity for all of these age groups is 100%, whereas those of the quantitative and qualitative RNA PCRs are 95.5 and 96.1% (5).

Our data showed that viral loads in infected infants were very high. This is consistent with other studies detecting the viral loads in children (4, 5, 9–11). Because of this usually high viral load in children, we recommend that a threshold of 5,000 IU/ml be used to interpret HIV-1 positivity, and we consider that cases below this value should be repeated to avoid false positivity. Our recommendation is twice lower than the one reported by Lilian et al. (10), because we found the NucliSENS very sensitive in this evaluation. Overall, NucliSENS EasyQ test appears to perform well for HIV-1 detection in children for high and medium viral load ranges. However, further studies need to be done to validate its sensitivity in low viral load categories.

HIV-1 RNA appeared to be less stable than DNA on the DBS, particularly at low viral load levels and at room temperature. For RNA tests, DBS samples should be stored at or below −20°C for long periods to avoid nucleic acid degradation.

Vertical transmission of HIV causes a high level of infant mortality. An early diagnosis of HIV infection in newborns is necessary to initiate therapy as early as possible. The NucliSENS EasyQ platform is a good alternative for the early diagnosis of HIV infection due to its high sensitivity and specificity. This platform indicates both patient infection status and the baseline viral load.

ACKNOWLEDGMENT

We acknowledge support from the Ministry of Health via the National Program against AIDS and STIs.

REFERENCES

1. **Cassol, S., et al.** 1994. Rapid screening for early detection of mother-to-child transmission of human immunodeficiency virus type 1. J. Clin. Microbiol. **32:**2641–2645.
2. **Cassol, S. A., et al.** 1992. Diagnosis of vertical HIV-1 transmission using the polymerase chain reaction and dried blood spot specimens. J. Acquir. Immune Defic. Syndr. **5:**113–119.
3. **Comeau, A. M., et al.** 1992. Polymerase chain reaction in detecting HIV infection among seropositive infants: relation to clinical status and age and to results of other assays. J. Acquir. Immune Defic. Syndr. **5:**271–278.
4. **Creek, T. L., et al.** 2007. Infant human immunodeficiency virus diagnosis in resource-limited settings: issues, technologies, and country experiences. Am. J. Obstet. Gynecol. **197:**S64–S71.
5. **Cunningham, C. K., et al.** 1999. Comparison of human immunodeficiency virus 1 DNA polymerase chain reaction and qualitative and quantitative RNA polymerase chain reaction in human immunodeficiency virus 1-exposed infants. Pediatr. Infect. Dis. J. **8:**30–35.
6. **De Rossi, A., et al.** 1991. Antigen detection, virus culture, polymerase chain reaction and in vitro antibody production in the diagnosis of vertically transmitted HIV-1 infection. AIDS **5:**15–20.
7. **Fransen, K., et al.** 1994. Design and evaluation of new, highly sensitive and specific primers for polymerase chain reaction detection of HIV-1-infected primary lymphocytes. Mol. Cell Probes **8:**317–322.
8. **Kane, C. T., et al.** 2008. Quantitation of HIV-1 RNA in dried blood spots by the real-time NucliSENS EasyQ HIV-1 assay in Senegal. J. Virol. Methods **148:**291–295.
9. **Leelawiwat, W., et al.** 2009. Dried blood spots for the diagnosis and quantitation of HIV-1: stability studies and evaluation of sensitivity and specificity for the diagnosis of infant HIV-1 infection in Thailand. J. Virol. Methods **155:**109–117.
10. **Lilian, R. R., K. Bhowan, and G. G. Sherman.** 2010. Early diagnosis of human immunodeficiency virus-1 infection in infants with the NucliSens EasyQ assay on dried blood spots. J. Clin. Virol. **48:**40–43.
11. **Lofgren, S. M., et al.** 2009. Evaluation of a dried blood spot HIV-1 RNA program for early infant diagnosis and viral load monitoring at rural and remote healthcare facilities. AIDS **23:**2459–2466.
12. **McIntosh, K., et al.** 1994. Blood culture in the first 6 months of life for the diagnosis of vertically transmitted human immunodeficiency virus infection. J. Infect. Dis. **170:**996–1000.
13. **Mehta, N., et al.** 2009. Low-cost HIV-1 diagnosis and quantification in dried blood spots by real-time PCR. PLoS One **4:**e5819.
14. **Nesheim, S., et al.** 1997. Diagnosis of perinatal human immunodeficiency virus infection by polymerase chain reaction and p24 antigen detection after immune complex dissociation in an urban community hospital. J. Infect. Dis. **175:**1333–1336.
15. **Ngo-Giang-Huong, N., et al.** 2008. Early HIV-1 diagnosis using in-house real-time PCR amplification on dried bloodspots for infants in remote and resource-limited settings. J. Acquir. Immune Defic. Syndr. **49:**465–471.
16. **Patton, J. C., et al.** 2007. Evaluation of dried whole blood spots obtained by heel or finger stick as an alternative to venous blood for diagnosis of human immunodeficiency virus type 1 infection in vertically exposed infants in the routine diagnostic laboratory. Clin. Vaccine Immunol. **14:**201–203.
17. **Piwowar-Manning, E., L. Lugalia, B. Kafufu, and J. B. Jackson.** 2008. Comparison of results obtained with Amplicor HIV-1 DNA PCR test version 1.5 using 100 versus 500 microliters of whole blood. J. Clin. Microbiol. **46:**1104–1105.
18. **Rakusan, T. A., R. H. Parrott, and J. L. Sever.** 1991. Limitations in the laboratory diagnosis of vertically acquired HIV infection. J. Acquir. Immune Defic. Syndr. **4:**116–121.
19. **Reigadas, S., M. H. Schrive, V. Aurillac-Lavignolle, and H. J. Fleury.** 2009. Quantitation of HIV-1 RNA in dried blood and plasma spots. J. Virol. Methods **161:**177–180.
20. **Rich, K. C., et al.** 1997. Immune complex-dissociated p24 antigen in congenital or perinatal HIV infection: role in the diagnosis and assessment of risk of infection in infants. J. Acquir. Immune Defic. Syndr. **12:**198–203.
21. **Rodes, B., et al.** 2007. Quantitative detection of plasma human immunodeficiency virus type 2 subtype A RNA by the NucliSENS EasyQ Assay (version 1.1). J. Clin. Microbiol. **45:**88–92.
22. **Rouet, F., et al.** 2001. Early diagnosis of paediatric HIV-1 infection among African breast-fed children using a quantitative plasma HIV RNA assay. AIDS **15:**1849–1856.
23. **Shermann, G. G., G. Stevens, S. A. Jones, P. Horsfield, and W. S. Stevens.** 2005. Dried blood spots improve access to VIH diagnosis and care for infants in low-resource setting. J. Acquir. Immune Defic. Syndr. **38:**615–617.
24. **Sherman, G. G., et al.** 2005. Polymerase chain reaction for diagnosis of human immunodeficiency virus infection in infants in low resource settings. Pediatr. Infect. Dis. J. **24:**993–997.
25. **Spira, R., et al.** 1999. Natural history of human immunodeficiency virus type 1 infection in children: a five-year prospective study in Rwanda. Pediatrics **104:**e56.
26. **Steketee, R. W., et al.** 1997. Early detection of perinatal human immunodeficiency virus (HIV) type 1 infection using HIV RNA amplification and detection. J. Infect. Dis. **175:**707–711.
27. **Stevens, W., L. Erasmus, M. Moloi, T. Taleng, and S. Sarang.** 2008. Performance of a novel human immunodeficiency virus (HIV) type 1 total nucleic acid-based real-time PCR assay using whole blood and dried blood spots for diagnosis of HIV in infants. J. Clin. Microbiol. **46:**3941–3945.
28. **Stevens, W., L. Wiggil, P. Horsfield, L. Coetzee, and L. E. Scott.** 2004. Evaluation of the NucliSENS EasyQ assay in HIV-1-infected individuals in South Africa. J. Virol. Methods **124:**105–110.
29. **Ugochukwu, E. F., and S. O. Kanu.** 2010. Early infant diagnosis of HIV infection in southeastern Nigeria: prevalence of HIV infection among HIV-exposed babies. West Afr. J. Med. **29:**3–7.
30. **UNAIDS.** 2010. UNAIDS report on the global AISD epidemic. UNAIDS, New York, NY. http://www.unaids.org/globalreport/global_report.htm.
31. **Violari, A., et al.** 2008. Early antiretroviral therapy and mortality among HIV-infected infants. N. Engl. J. Med. **359:**2233–2244.
32. **Young, N. L., et al.** 2000. Early diagnosis of HIV-1-infected infants in Thailand using RNA and DNA PCR assays sensitive to non-B subtypes. J. Acquir. Immune Defic. Syndr. **24:**401–407.