Adaptation of the Ultrasensitive HIV-1 p24 Antigen Assay to Dried Blood Spot Testing

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Summary: Implementation of molecular tests for the assessment of pediatric HIV-1 infection in resource-limited countries is difficult because of technical complexity and costs. Alternatives like the ultrasensitive HIV-1 p24 antigen enzyme-linked immunosorbent assay have therefore been proposed. We have now adapted this test to dried blood spot (DBS) plasma p24 antigen (p24). High background activity was recognized as originating from endogenous peroxidase and eliminated by H2O2 quenching. The assay was evaluated with 72 pediatric specimens from Tanzania and with 210 pediatric or adult specimens from Switzerland. A real-time polymerase chain reaction assay for DBS DNA and/or plasma RNA identified HIV-1 infection in 38 Tanzanian children. HIV-1 subtypes included 18 C, 9 A1, 8 D, 1 AC, 1 J-like, and 1 unidentified. The detection rates for the different assays were as follows: DBS-p24, 32 (84%) of 38 samples; DBS DNA, 30 (79%) of 38 samples; plasma-p24, 23 (85%) of 27 samples; and plasma RNA, 30 (100%) of 30 samples. False-negative DBS-p24 was associated with subtype D (R² = 0.83, P < 0.0001). DBS-p24 detection for non-D subtypes was 93% (95% confidence interval: 81% to 99%), and for subtype C, it was 94% (95% confidence interval: 76% to 99%). Specificity among 193 HIV-negative DBS samples was 100%. Correlation of DBS-p24 and plasma-p24 concentrations was excellent (R² = 0.83, P < 0.0001). DBS-p24 is thus a promising alternative to molecular tests for HIV-1 in subtype C regions. It should now be evaluated in large studies of children for accurate assessment of diagnostic sensitivity.

Key Words: dried blood spot, HIV-1 p24 antigen, pediatric HIV-1 diagnosis, plasma HIV-1 RNA, real-time DBS DNA polymerase chain reaction, resource-poor settings

An estimated 5.9 million children have been infected with HIV-1 since the beginning of the pandemic, 90% of them in Africa. In the absence of preventive measures, 13% to 42% of children born to HIV-infected mothers acquire HIV-1 in developing countries. Therefore, strategies to prevent mother-to-child transmission have been introduced in most countries and are successful in keeping the transmission rate low. Nevertheless, the diagnosis of pediatric HIV-1 infection and the monitoring of HIV-infected children remain a challenge. The HIV-1 DNA or RNA tests that are required for these tasks are costly, technically complex, and rarely available in resource-constrained countries. Serologic tests are not suited for neonates/infants younger than 18 months of age because they do not discriminate between maternally transferred antibodies and self-response to infection. As emphasized in the United Nations millennium development goals, straightforward and less expensive tests combined with simplified preanalytic procedures are urgently needed for HIV-1 diagnosis in children. In this respect, the use of dried blood spot (DBS) samples on filter papers has proven to be a convenient sampling technique suitable for remote district hospitals and rural settings.1-3

Our group has previously developed a quantitative and ultrasensitive HIV-1 plasma p24 antigen (p24) assay that is inexpensive, easy to perform, and does not require large and expensive equipment.4-6 This enzyme-linked immunosorbent assay (ELISA)-based procedure is characterized by an efficient HIV-1 disruption step,7,8 heat-mediated destruction of interfering antibodies,9 and a signal amplification step.10 Provided that the respective HIV-1 subtype is recognized optimally, the accumulated evidence demonstrates that the p24 assay is a valid and inexpensive alternative to tests detecting HIV-1 RNA.11-13 Specifically, in HIV-1 subtype B and C settings, p24-based diagnosis of pediatric HIV-1 infection was found to be similarly sensitive and specific as polymerase chain reaction (PCR)-based measurement of HIV-1 DNA or RNA.14,15 Furthermore, p24 was equivalent to HIV-1 RNA in predicting short-term CD4+ T-cell changes in prospectively followed children.16

We report the adaptation of the p24 assay to DBS samples and illustrate its suitability for measuring HIV-1 p24 antigen in children. The use of DBS samples should particularly benefit children in rural settings and has the potential of greatly improving pediatric HIV-1 care in resource-constrained countries.
PATIENTS AND METHODS
Study Participants and Specimens
Seventy-two infants, children, and adolescents between 1 month and 15 years of age (median = 26 months, mean = 49 months) living in different rural areas of Ifakara, Tanzania were investigated. HIV screening tests (Capillus; Trinity Biotech PLC, Wicklow, Ireland) yielded a positive result in 30 subjects, whereas the results in 13 were negative. No serology was performed on 29 subjects, with 23 of them being too young (<18 months of age). Blood specimens were collected after approval of the study by the Ifakara Health Research and Development Center Institutional Review Board and after obtaining informed consent from the children’s parents or legal guardians. Ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood was collected by venipuncture; capillary blood was collected using single-use disposable lancets. One to 3 100-μL aliquots of venous blood were applied to Whatman filter paper no. 3 (Whatman International Ltd, Maidstone England), whereas the remainder was centrifuged for plasma harvest. The filters were dried for at least 3 hours at room temperature and were stored in plastic bags.

For comparison, 47 blood samples from 42 HIV-1 RNA-positive adults visiting the HIV-1 Outpatient Clinic of the Zurich University Hospital, Switzerland, and 15 blood samples, mostly capillary, from 9 HIV-1-infected children cared for at the Zurich University Children’s Hospital Outpatient Clinic were collected. Similarly, blood samples from 100 adult healthy blood donors seen at the Zurich Swiss Red Cross Blood donation center and from 59 uninfected children from the Zurich University Children’s Hospital were used. Of the 9 HIV-1–infected Swiss children, 3 were infected with HIV-1 subtype B, 1 was infected with HIV-1 GD recombinant, and the subtypes of 5 children were unknown. The study was approved by the Ethical Committee of the University Children’s Hospital of Zurich. DBS samples in Switzerland were prepared by using filter paper (S&S no. 903; Schleicher & Schuell, Basel, Switzerland), dried, and stored in plastic bags with a desiccant pack at 4°C.

For preparation of quantitative p24 standards for DBS testing, p24 antigen was mixed into HIV-negative EDTA-anticoagulated blood of a healthy volunteer and serially diluted to yield concentrations extending from 1000 to 0.15 pg/mL.

Sample Pretreatment for HIV-1 p24 Assay
DBS disks 16 mm in diameter were cut out, transferred to Eppendorf tubes, and washed in 10 mM of Tris HCl and 1 mM of EDTA for 30 minutes. The supernatants were removed, and 2 more quick washes followed. Three hundred microliters of a buffer containing 10 mM of Tris HCl (pH 8.0), 50 mM of potassium chloride (KCl), 0.45% Tween 20, 0.45% NP-40, and 1.3 mg/mL of proteinase K were added. The tubes were incubated at 56°C for 30 minutes and at 90°C for 20 minutes in an Eppendorf thermomixer with agitation. The mixture with the filters was subsequently transferred to a QiaShredder column (Qiagen, Hilden, Germany) and spun for 1 minute at 20,800g. Three hundred microliters of buffer AL (Qiagen) was added and spun as described previously. A matched amount of absolute alcohol was added to the filtrate and applied to a QiAamp Spin column (Qiagen). The extraction then followed the procedure of the Qiagen protocol, and the DNA was finally eluted in 55 μL of buffer AE (Qiagen). For each extraction batch, DBS spiked with 500 copies of plasmid pBT1 (pBT1) and DBS from healthy individuals were used as controls and amplified with the patient samples.

For real-time PCR, 100-μL reactions were set up, with 50-μL DNA input. Primers and reaction conditions corresponded to those described previously, except that Taq DNA polymerase was replaced by a heat-activated version of the enzyme and 0.04% SYBRgreen (Synergy Brands, Inc., Chicago, IL) and 250 nM of a tetramethyl-6-carboxyrhodamine dye (TAMRA)-labeled (dT)6 reference oligonucleotide virus disruption buffer at the end of the day. Plasma specimens were treated as previously described.

Testing for HIV-1 p24 Antigen
HIV-1 p24 antigen was measured using the HIV-1 p24 ELISA kit in combination with the ELAST Amplification System (both from PerkinElmer Life Sciences, Boston, MA). Duplicate 250-μL aliquots of each pretreated specimen (DBS eluate or plasma) were transferred to the ELISA plate and processed as previously described.

For the DBS eluates, the plates were incubated overnight at 37°C and the plasma protocol was followed, with minor modifications as follows. After the first wash, we introduced a peroxidase quenching step consisting of a 10-minute incubation at 37°C with 0.3% H2O2 in 50 mM of Tris HCl, pH 7.4 to 7.8. After washing, 100 μL of biotinylated detector antibody was incubated at 37°C for 75 minutes. Finally, 100 μL of streptavidin-horseradish peroxidase (HRP) diluted at a 1:25 ratio was added for 15 minutes at 37°C.

The concentration of p24 was determined by means of a simple Microsoft Excel–based (Microsoft, Wallisellen, Switzerland) program that is available free of cost from the authors. Based on the optical density values of the 9 quantitative virus samples, mostly capillary, from 9 HIV-1–infected children car...
were added for amplicon detection. Cycling was as follows: 10 minutes at 95°C, 10 cycles of 15 seconds at 95°C, 30 seconds at 65°C, 30 seconds at 72°C, and 55 cycles of 15 seconds at 90°C and 1 minute at 60°C. A dissociation curve was performed after each run for ensuring specificity of the amplicon.

**Real-time Polymerase Chain Reaction for HIV-1 RNA**

RNA was extracted using the reagents of the Amplicor HIV-1 Monitor assay, version 1.5 (Roche Molecular Diagnostics, Rotkreuz, Switzerland). Quantitative real-time RT-PCR was carried out as described but with primers targeting the viral pol gene. To estimate HIV-1 RNA concentrations, an external RNA standard was included in each run, which was created by spiking normal human donor plasma with known amounts of virus particles. This virus had been calibrated before by using the Amplicor HIV-1 Monitor Assay, version 1.5. The estimated limit of detection of this assay was 100 copies/mL. To exclude loss of viral RNA or reverse transcriptase (RT)-PCR inhibition, all plasma specimens were spiked with feline immunodeficiency virus (FIV) before RNA extraction and also tested by real-time RT-PCR for FIV RNA using identical reaction conditions. No significant inhibition of the RT-PCR assay was observed.

**HIV-1 Subtype Identification**

For genotypic subtyping, the region of the gag gene encoding p24 was amplified by RT-PCR or, if plasma was unavailable, by nested PCR using proviral DNA extracted from DBS. The presence or absence of amplified DNA was verified by agarose-gel electrophoresis. Five microliters of the DNA-containing reaction mixture was then treated with ExoSap-IT (Amersham Biosciences, Otelfingen, Switzerland), diluted, and sequenced by cycle sequencing with the respective amplification primers and the dye terminator chemistry (Applied Biosystems, Rotkreuz, Switzerland). The resulting products were analyzed on an ABI 3100 DNA sequencer (Applied Biosystems). Sequences were assembled and edited using Autoassembler software (Applied Biosystems). For subtyping, the HIV genotyping tool of the National Center for Biotechnology Information was used (available at: http://www.ncbi.nlm.nih.gov/projects/genotyping). In addition, DNA alignments and phylogenetic trees were generated with Mac Vector 7.5 (Accelrys, Cambridge, England).

**Data Evaluation and Statistics**

For all analyses, the StatView version 5.0 program for Macintosh (SAS Institute, Cary, NC) was used. The Spearman rank correlation and Pearson correlation were used for correlation analysis. Logistic regression analysis was used to identify nominal or continuous variables influencing a nominal outcome variable. All concentrations were evaluated as log_{10} transformed values. The p24 antigen concentrations, which directly represent experimentally determined variables, were entered as calculated based on the standard curve, irrespective of whether they were above or below the cutoff for negativity.

**RESULTS**

Testing of DBS eluates from HIV-negative controls resulted in a significant signal in the HIV-1 p24 ELISA even if the assay steps involving incubation with the kit's streptavidin-HRP were omitted (data not shown). We suspected that this high background might be attributable to the persistence of endogeneous peroxidase activity in the DBS eluates; hence, we evaluated the introduction of a peroxidase-quenching step after sample incubation. Treatment with 0.3% H₂O₂ reduced the background signal seen with HIV-negative controls approximately 10-fold (Fig. 1A), without impeding on the signal generated by HIV-1–positive samples (see Fig. 1B).

We assessed the potential of this method on a group of 72 treatment-naive children living in the rural area of Ifakara, Tanzania (Table 1). A total of 38 subjects were identified as HIV-1 infected using the DBS HIV-1 DNA PCR assay (n = 72) and/or RT-PCR assay for HIV-1 RNA in plasma (n = 30). Among this group, 18 subjects were infected with subtype C, 9 with subtype A1, 8 with subtype D, 1 with a J-like subtype, 1 with a C/A recombinant, and 1 with an unidentified subtype.

**FIGURE 1.** Background reduction by means of peroxidase quenching. (A) Eight HIV-negative control samples were tested in parallel without or with, respectively, a quenching step (see Methods section). Bars indicate the mean optical density units and the SD of duplicate sample testing. (B) Quenching does not impair the signal generated by HIV-1–positive samples of different p24 content (single testing).
Overall, these results confirm previous assessments on Tanzanian HIV-1 subtypes. The repartition of the subtypes by age categories was as follows: the category of age 0 to 18 months (n = 7) had 3 neonates/infants infected with subtype D, 2 with subtype C, 1 with subtype A1, and 1 with an unknown subtype; the category of age 21 to 36 months (n = 8) had 3 infants/children infected with subtype D, 3 with subtype C, and 2 with subtype A1; the category of age 37 to 72 months (n = 8) had 6 children infected with subtype C and 2 with subtype A1; and the category of age 84 to 180 months (n = 14) had 2 children/adolescents infected with subtype D, 6 with subtype C, 4 with subtype A1, 1 with CA recombinant, and 1 with J-like subtype. HIV-1 p24 testing of DBS eluates identified 32 of these 38 infected subjects as positive (84%) and 1 additional subject as indeterminate, although scoring 5 samples as (false) negative (13%). A DNA PCR assay of DBS scored 30 samples as positive (79%) and 8 falsely as negative (21%). Testing of frozen plasma samples, which were not available from all 38 infected subjects yielded a similar rate of plasma p24 positivity (85%), whereas all the 30 HIV-infected subjects tested were positive by an RT-PCR assay of plasma samples (100%).

Table 2. Overall, these results confirm previous assessments on Tanzanian HIV-1 subtypes. The repartition of the subtypes by age categories was as follows: the category of age 0 to 18 months (n = 7) had 3 neonates/infants infected with subtype D, 2 with subtype C, 1 with subtype A1, and 1 with an unknown subtype; the category of age 21 to 36 months (n = 8) had 3 infants/children infected with subtype D, 3 with subtype C, and 2 with subtype A1; the category of age 37 to 72 months (n = 8) had 6 children infected with subtype C and 2 with subtype A1; and the category of age 84 to 180 months (n = 14) had 2 children/adolescents infected with subtype D, 6 with subtype C, 4 with subtype A1, 1 with CA recombinant, and 1 with J-like subtype. HIV-1 p24 testing of DBS eluates identified 32 of these 38 infected subjects as positive (84%) and 1 additional subject as indeterminate, although scoring 5 samples as (false) negative (13%). A DNA PCR assay of DBS scored 30 samples as positive (79%) and 8 falsely as negative (21%). Testing of frozen plasma samples, which were not available from all 38 infected subjects yielded a similar rate of plasma p24 positivity (85%), whereas all the 30 HIV-infected subjects tested were positive by an RT-PCR assay of plasma samples (100%).

When breaking down the sensitivity by HIV-1 subtype, the DBS p24 assay showed good sensitivity for all but subtype

### TABLE 1. Characteristics of the Study Participants

<table>
<thead>
<tr>
<th></th>
<th>A. Tanzanian HIV-Infected Pediatric Subjects (n = 38)</th>
<th>B. Swiss HIV-Infected Children (n = 34, 52 data points)</th>
<th>C. Swiss HIV-Infected Adults (n = 42)</th>
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<tbody>
<tr>
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<td><strong>B. Swiss HIV-Infected Children (n = 34, 52 data points)</strong></td>
<td><strong>C. Swiss HIV-Infected Adults (n = 42)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Age (mo)</strong></td>
<td>n = 38</td>
<td>n = 31</td>
<td>n = 42</td>
</tr>
<tr>
<td><strong>HIV-1 RNA (copies/mL)</strong></td>
<td>Median: 30*</td>
<td>Median: 15*</td>
<td>Median: 47*</td>
</tr>
<tr>
<td><strong>Plasma HIV-1 p24 (fg/mL)</strong></td>
<td>Median: 29†</td>
<td>Median: 12*</td>
<td>Median: 41*</td>
</tr>
<tr>
<td><strong>CD4+ T-cell counts/μL</strong></td>
<td>Median: 15‡</td>
<td>Median: nd</td>
<td>Median: nd</td>
</tr>
<tr>
<td></td>
<td>Interquartile Range: 164–381</td>
<td>Interquartile Range: nd</td>
<td>Interquartile Range: nd</td>
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</table>

*No plasma was available for 8 samples.
†One sample did not have enough plasma.
‡CD4+ T-cell counts were not available for 23 samples.

### TABLE 2. Diagnostic Sensitivities of Different Tests Applied to DBS or Plasma from 38 HIV-Infected Tanzanian Pediatric Subjects

<table>
<thead>
<tr>
<th>HIV-1 Subtype</th>
<th>n (%)</th>
<th>Tests on DBS</th>
<th>Tests on Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>[no. positive/no. tested] (%)</td>
<td>[no. positive/no. tested] (%)</td>
</tr>
<tr>
<td>HIV-1 DNA</td>
<td></td>
<td>HIV-1 p24</td>
<td>HIV-1 RNA</td>
</tr>
<tr>
<td>HIV-1 p24</td>
<td></td>
<td>HIV-1 p24</td>
<td>HIV-1 p24</td>
</tr>
</tbody>
</table>

HIV-1 infection status was identified based on a positive result in PCR for HIV-1 RNA and/or HIV-1 DNA.
*HIV-1 RNA in the 1 missed sample from a 24-month-old child was 389 copies/mL.
†Undetected sample was from a 12-month-old infant with an indeterminate result (see Methods section).
nd indicates not determined.
The recognition rate for DBS-p24 in subtype C was 94.4%, with only 1 sample scoring negative for p24. This child was 24 months old, with an HIV-1 RNA concentration of only 389 copies/mL and an undetectable HIV-1 DNA content. Detection of DBS-p24 was only 50% for subtype D (4 of 8 samples), whereas it was 93% for non-D (28 of 30 samples). Of note, the 3 infected subjects ≤ 18 months of age with subtype D were correctly recognized, whereas 4 of 5 infected subjects older than 18 months of age were not detected by DBS-p24. Logistic regression analysis for the influence of subtype D versus non-D, gender, and age on positivity of DBS-p24 showed that subtype D was associated with a significantly lowered probability of positivity (Table 3).

Surprisingly, the PCR assay for HIV-1 DNA scored all subtype D samples positive but missed 8 of the 30 non-D samples. Sequence analysis encompassing the p24 region of 7 of these non-D subtype samples, all of which were positive for DBS-p24, did not reveal mismatches that could explain an amplification failure. Similarly, control reactions disclosed no evidence for inhibition of the amplification reaction. Amplification of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase yielded copy numbers that were compatible with normal content and quality of the DNA (data not shown).

We next assessed the correlation of DBS-p24 with plasma parameters (ie, plasma-p24 and HIV-1 RNA). We evaluated the results according to ethnic background to account for differences in p24 production or dynamics between the populations. There was an overall excellent correlation between DBS-p24 and plasma-p24 measurements (Fig. 2A), and samples from Tanzania and Switzerland were similar ($R^2 = 0.840$ and $R^2 = 0.835$, respectively; $P < 0.0001$ for both). Likewise, a significant but less impressive correlation was observed with the concentration of HIV-1 RNA (see Fig. 2B). There was a significant correlation (Pearson $P = 0.012$, Spearman $P = 0.06$) for the 23 Tanzanian samples with a non-D subtype, and their regression curve was similar to that of the 62 Swiss samples.

Specificity was assessed using Tanzanian and Swiss specimens from sample pediatric and adult populations. All the samples from the 59 HIV-1–negative Swiss children and the 100 Swiss adult blood donors as well as those from the 34 HIV-1–negative Tanzanian children, 193 in total, were negative by DBS-p24, thus demonstrating a 100% diagnostic specificity.

We finally compared the cutoff values of plasma-p24 and DBS-p24 based on the 34 HIV-1–negative Tanzanian specimens. In this African setting, the cutoff for p24 positivity was calculated as corresponding to 3.5 pg/mL for plasma-p24.

### Table 3: Multivariate Logistic Regression Analysis of Parameters Influencing Positivity in the DBS-p24 Assay

<table>
<thead>
<tr>
<th>Variable</th>
<th>$P$</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>$X^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, per month</td>
<td>0.76</td>
<td>0.997</td>
<td>0.975 to 1.019</td>
<td>0.091</td>
<td>0.76</td>
</tr>
<tr>
<td>Gender</td>
<td>0.40</td>
<td>0.398</td>
<td>0.046 to 3.426</td>
<td>0.725</td>
<td>0.39</td>
</tr>
<tr>
<td>Subtype D versus non-D</td>
<td>0.012</td>
<td>0.070</td>
<td>0.009 to 0.561</td>
<td>7.05</td>
<td>0.008</td>
</tr>
</tbody>
</table>

FIGURE 2. Correlation of DBS-p24 with plasma-p24 and HIV-1 RNA. (A) DBS-p24 independent of plasma-p24 in the 27 Tanzanian (TZ) subjects and 62 Swiss positive controls (Swiss) in whom both tests were performed. The linear regression curves are shown at the top of the panel. (B) DBS-p24 independent of HIV-1 RNA concentration and HIV-1 subtype for Tanzanian (TZ) subjects and Swiss positive controls (Swiss). Each subtype is identified by symbols explained at the bottom of the panel. The linear regression curve equations, separated for Tanzania non-D (TZ, non-D; n = 23), Tanzanian D (TZ, D; n = 7), and Swiss samples (Swiss; n = 62), are shown at the top. $P$ values shown were determined by the Pearson correlation assay. One of the 8 subtype D samples is not shown, because there was no result for HIV-1 RNA. n.d. indicates “not determined”.

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and to 4.2 pg/mL for DBS-p24. Likewise, the cutoff for p24 negativity was at 2.3 pg/mL for plasma-p24 and at 3.0 pg/mL for DBS-p24. Thus, the DBS-p24 method displays comparable analytic sensitivity to the plasma-p24 method.

**DISCUSSION**

The assessment of a child’s HIV-1 status early in life and the monitoring of that child’s treatment are unresolved key issues that affect the rolling out of antiretroviral treatment (ART).

We adapted the HIV-1 p24 assay to DBS samples and considerably reduced the known high background in DBS samples by introducing a peroxidase quenching step. This resulted in a sensitivity that was overall equal to that observed with plasma-p24 and equal to that achieved on these samples with a real-time PCR method for DBS HIV-1 DNA.

Results obtained with the DBS-p24 assay were best for HIV-1 subtype C, which is in agreement with findings from larger studies conducted in Zimbabwe and South Africa. In addition, despite an open-bench pretreatment area, the assay exhibited a diagnostic specificity of 100%. Patton et al. recently published a method for DBS-p24 testing using a modified protocol of the p24 ultrasensitive assay. They reported a diagnostic specificity of 98.8% and a specificity of 100% in a subtype C endemic region. Our 94% detection rate achieved with the 18 subtype C samples is thus similar; however, our sample population was generally tested after the time of primary infection (ie, after the viral load peaks).

Another difference between the 2 methods is the handling of standards. Their use of washed erythrocyte concentrates for the standards may not be representative of true DBS samples, because white blood cells and platelets as the likely sources of endogenous peroxidase activity were largely removed.

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Monitoring pediatric HIV-1 infection. Because the peak viral loads of pediatric HIV-1 infection are observed between 6 weeks and 3 months of age, the p24 assay may well be more sensitive when evaluated in this age group compared with the present study, in which the subjects were generally older. Based on our results, the test, as presented now, is particularly promising in subtype C areas like southern Africa, China, and India. Our DBS-p24 method should, however, also detect most pediatric HIV-1 infections in East African countries, where a variety of other HIV-1 subtypes and recombinant forms may be present. It must be stressed, however, that more than 90% of all infected children worldwide are living in sub-Saharan Africa, where subtype C is predominant (Joint United Nations Program on HIV/AIDS [UNAIDS], December 2005; 2.1 million in sub-Saharan Africa of 2.3 million total).

As a practical guideline, whenever a pediatric HIV-1 infection is to be diagnosed, a simple antibody assay such as the Capillus should be done first. A positive result of such a test in an infant, child, or adolescent older than 18 months indicates HIV-1 infection. In younger neonates and infants, a positive antibody test result only reflects exposure to maternal HIV infection, and a test for a viral component such as HIV-1 DNA, HIV-1 RNA, or, as we propose, HIV-1 p24 has to be performed to prove the infection. A positive result in the DBS-p24 assay implies a high probability for HIV-1 infection. In a subtype C setting, a child with a negative DBS-p24 result tested at approximately 3 months of age is unlikely to be infected, because others have found a high sensitivity under such circumstances. In areas of mixed subtypes, the possibility of a nondetectable subtype should be considered and p24-negative infants should be checked again for HIV antibodies at the age of 18 months.

Larger clinical studies must now be initiated, and the test must be further adapted to recognize all HIV-1 subtypes and isolates. Although the solution proposed here still offers room for improvement, it constitutes an important advancement toward state-of-the-art diagnosis and treatment of pediatric HIV-1 infection in low-resource countries that should assist in effective provision of ART in peripheral settings.

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