Potential of a Simplified p24 Assay for the Early Diagnosis of Infant HIV-1 Infection in Haiti

Erik George 1*, Carole Anne Beauharnais 2, Emilio Brignoli 2, Francine Noel 2, Gyrlande Bois 2, Patricia De Matteis Rouzier 2, Martine Altenor 2, Daniel Lauture 2, Marlène Hosty 3, Sapna Mehta 4, Peter F. Wright 4, Jean W. Pape 2

From the Department of Medicine, Division of International Medicine and Infectious Diseases, Weill Medical College of Cornell University, New York, NY 1, the Groupe Haitien d’Etude du Sarcome de Kaposi et des Infections Opportunistes (GHESKIO), Port-au-Prince, Haiti 2, the Hopital Immaculeé Conception des Cayes, Les Cayes, Haiti 3, and the Department of Pediatrics, Division of Pediatric Infectious Diseases, Vanderbilt University School of Medicine, Nashville, TN 4

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*Correspondence and reprint requests to:

Dr. Erik George

Hudson Infectious Disease Associates PC

540 North State Road

Briarcliff Manor, NY 10510-1598

Tel 914 762-2276

Fax: 914 762-2894
ABSTRACT

With global efforts to scale-up prevention of mother-to-child transmission services and pediatric antiretroviral therapy, there is an urgent need to introduce a simple low-cost infant HIV test in the field. We postulated that the p24 antigen capture ELISA could be simplified by eliminating signal amplification, without compromising on diagnostic accuracy.

NOTE

In 2006, 530,000 children contracted HIV-1 infection worldwide, and the vast majority resided in low-income countries (11)(14)(29). In high-income countries, prompt diagnosis and early introduction of antiretroviral therapy (ART) have dramatically reduced mortality in children infected with HIV (10). Nucleic acid sequence based amplification assays, the gold standard for infant HIV diagnosis, are costly and technologically complex making their use in poor countries unfeasible (3)(4)(6)(20).

Lately, improved accuracy of commercially available p24 antigen microelisa kits have been demonstrated with more efficient solid-phase antigen capture and buffer systems, coupled with signal amplification ELISA reducing lower limit of p24 antigen detection from 10 to 0.5 pg/ml (8)(16)(18)(22-25). The ultrasensitive p24 assay - validated in countries where HIV subtypes A, B, C, D, or E predominate (7)(13)(15)(19) - has achieved sensitivity of 92-100% in pediatric cohorts at a cost of approximately $7 per test (12)(13)(27)(30).

The objective of this study was to determine the performance of two commercially available p24 antigen assays testing perinatally HIV-1 exposed infants in Haiti: the HIV-
Antigen Microelisa System Vironostika Assay (bioMérieux, Boxtel) with (Up24) and without (Sp24) the addition of the signal amplification system Enzyme-linked Amplified Sorbent Test (ELAST, PerkinElmer Life Science Inc, Boston, MA) (17), and the automated VIDAS HIV p24 II Assay (bioMérieux, Marcy l’Etoile). The studies were conducted at the GHESKIO Centers in Port-au-Prince and the district hospital Immaculée Conception in Les Cayes between April 2005 and October 2006. Care-giver consent was obtained for routine HIV testing of all subjects. The Internal Review Boards of GHESKIO and Weill Medical College of Cornell University approved these studies. The reference method was the Real Time RNA Assay NucliSens EasyQ HIV-1 version 1.1 (bioMérieux, Boxtel) (5). In blinded cross-sectional studies, 401 frozen plasma samples were analyzed of 233 HIV-1 subtype B exposed ART naïve infants (mean age: 2.8 months old, interquartile range (IQR) 0.2-3.6) consecutively enrolled at the study centers. The plasma samples were obtained by venipuncture and stored at –70°C. The manufacturer’s instructions conducting p24 antigen testing with the respective assays were followed. However, the following modifications of the Up24 and Sp24 assays were made: 1) The entire testing procedure was performed at room temperature (24-28°C) - except antigen-antibody heat denaturation (100ºC, 5 minutes) 2) Plasma samples were diluted 1:5 with virus disruption buffer (0.5% Triton X-100 and 2.9% sodium dodecyl sulfate). In addition antigen solid-phase incubation time of the Sp24 assay was prolonged to 16 hours (9). In the initial comparison, the performance of the Up24 versus the VIDAS assay to HIV-1 RNA quantification was documented analyzing 200 plasma samples. In a second comparison, the performance of the Sp24 assay to HIV-1 RNA quantification was documented on another 201 plasma samples. For each assay the optical density (OD)
values at 450nm readings were recorded to calculate the p24 antigen concentration against a standard curve (cut-off value=mean absorbance of 3 negative controls + 3SD).

The sensitivity of the Up24, Sp24, and VIDAS assays testing plasma of our infant population were: 93% (95% confidence interval (CI): 85-100%), 91% (CI: 83-100%), and 95% (CI: 89-100%), respectively, (P=0.44). Detection of p24 antigen with the Sp24 assay failed in four infants aged 0 day, 2 day, 10 days, and 3 months with HIV-1 RNA levels of 3.2, 3.6, 3.1, and 3.2 log_{10} copies/ml. Detection of p24 antigen with the Sp24 assay was successful in 11/15 (73%) samples with HIV-1 RNA levels < 4.0 log_{10} copies/ml, and in 30/30 (100%) samples with levels > 4.0 log_{10} copies/ml. The correlations of HIV-1 RNA (log_{10} copies/ml) and p24 antigen (log_{10} fg/ml) quantification by Spearman rank analysis were: Up24 r=0.567 (R²=0.321, P<0.01; n=41), Sp24 r=0.590 (R²=0.348, P<0.01; n=40), and VIDAS r=0.704 (R²=0.495, P<0.01; n=41). The specificity of the Up24, Sp24, and VIDAS assays were: 99% (CI: 98-100%), 97% (CI: 94-100%), 99% (CI: 98-100%), respectively, (P=0.10). The diagnostic range of the Sp24 assay was within a range of 3.0-80 pg/ml. Sp24 intra-assay signal variability (CV%=extreme OD/mean OD) of five repeats of the same plasma with the follow p24 antigen concentrations 0, 7, 24, 59 pg/ml were: 14% (CI: 0-28%), 23% (CI: 9-37%), 21% (CI: 12-30%), and 21% (CI: 13-29%), respectively. Sp24 inter-assay signal variability at three consecutive trials of plasma samples with the follow p24 antigen concentrations 0, 1, 5, 20, 80 pg/ml were: 8% (CI: 0-18%), 24% (CI: 15-33%), 22% (CI: 14-30%), 14% (CI: 8-20%), and 4% (CI: 0-10%).

Previous decision analysis modeling, assuming 5% ART availability, has estimated that a pediatric HIV test, could benefit risk populations in terms of total adjusted life years.
saved if it achieves at least 90% sensitivity and specificity (1). We explored the utility of
different p24 assays for infant HIV-1 testing in Haiti. A simplified protocol applying
overnight (16h) p24 antigen solid-phase incubation ELISA without added signal
amplification attained comparable diagnostic accuracy to the Up24 and the VIDAS
assays. Detection of p24 antigen with the Sp24 assay was successful in all 30/30 (100%) samples with HIV-1 RNA levels > 4.0 log_{10} copies/ml (18). The mean HIV-1 RNA level of the infected cohort was 5.7 (IQR 5.3-6.0) log_{10} copies/ml – approximately 1 log_{10}
copies/ml greater than that observed in adults (21)(26). We conclude that signal
amplification of p24 antigen ELISA was redundant testing HIV-exposed infants in the
perinatal period, when plasma viral concentrations reach levels > 5.0 log_{10} copies/ml
generally within weeks of infection regardless of mode of transmission. Storing,
preparing, diluting, and pipetting reagents for signal amplification is eliminated, reducing
effective processing time by 90 minutes, minimizing person-to-person variability, and
cutting expenses in nearly half. We believe even greater diagnostic accuracy may be
achievable in a relatively older population (mean age of our cohort: 2.8 months, IQR 0.2-
3.6) if routing testing is conducted at 4 weeks of age and older, and freeze-thaw cycles
and storage time of plasma are minimized (1)(2)(28). Importantly, we set up Sp24 assay
testing in the district hospital in Les Cayes. The local technician trained obtained
comparable accuracy with the central laboratory in Port-au-Prince. The Sp24 assay
technique yielded inter-assay signal variability of 16%. Repeat testing of samples falling
close to cut-off level (OD within the range cut-off +/- 0.1 (2SD)) is crucial. Providers
also need to monitor and test HIV exposed infants over time.
The simplified p24 assay achieved significant diagnostic accuracy in settings with minimal infrastructure. We strongly encourage efforts to accelerate development of point-of-care diagnostic tests for use in resource-limited settings. In the interim period, utilization of the low-cost assays such as the Sp24 assay will be critical to capitalize on the momentum in the fight to control the global AIDS pandemic.

FOOTNOTES

Author Emilio Brignoli was during his affiliation with the GHESKIO Centers employed by the Fondation Rodolphe Mérieux.

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the relative contributions of intra-uterine and intra-partum transmission. Aids 9:F7-11.


immunodeficiency virus type 1 RNA viral load assay in resource-limited settings.


<table>
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<tr>
<th>p24 Antigen Assay</th>
<th>Positive</th>
<th>Negative</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tr>
<td>Vironostika + ELAST (^{a,b,c})</td>
<td>40</td>
<td>1</td>
<td>93% (95% CI: 85-100%)</td>
<td>99% (95% CI: 98-100%)</td>
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<td>3</td>
<td>156</td>
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<tr>
<td>Vironostika (^{b,d})</td>
<td>41</td>
<td>5</td>
<td>91% (95% CI: 83-100%)</td>
<td>97% (95% CI: 94-100%)</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>151</td>
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<td></td>
</tr>
<tr>
<td>VIDAS (^{c,e})</td>
<td>41</td>
<td>1</td>
<td>95% (95% CI: 89-100%)</td>
<td>99% (95% CI: 98-100%)</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>156</td>
<td></td>
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</tbody>
</table>


\(^a\) ELAST ELISA Amplification System - PerkinElmer Life Science Inc, Boston, MA, USA

\(^b\) Vironostika - HIV-1 p24 Antigen Microelisa System Vironostika - bioMérieux, Boxtel, Netherlands

\(^c\) Trial 1 testing, n=200 (April 1, 2005, and June 30, 2005)

\(^d\) Trial 2 testing, n=201 (July 1, 2006, and October 1, 2006)

\(^e\) VIDAS HIV p24 II Assay - bioMérieux, Boxtel, Netherlands
FIGURE LEGENDS

TABLE 1. The diagnostic performance of p24 antigen assays in Haiti.
ERRATUM

Potential of a Simplified p24 Assay for Early Diagnosis of Infant Human Immunodeficiency Virus Type 1 Infection in Haiti

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Department of Medicine, Division of International Medicine and Infectious Diseases, Weill Medical College of Cornell University, New York, New York; Groupe Haitien d’Etude du Sarcome de Kaposi et des Infections Opportunistes (GHESKIO), Port-au-Prince, Haiti; Hopital immaculée Conception des Cayes, Les Cayes, Haiti; and Department of Pediatrics, Division of Pediatric Infectious Diseases, Vanderbilt University School of Medicine, Nashville, Tennessee

Volume 45, no. 10, p. 3416–3418, 2007. Page 3416, column 2, line 9: “0.2 to 3.6 years old” should read “0.2 to 3.6 months old.”