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Short communication

Ultrasensitive quantitative HIV-1 p24 antigen assay adapted to dried plasma spots to improve treatment monitoring in low-resource settings

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Abstract

Background: Our group has previously developed a quantitative and ultrasensitive HIV-1 p24 antigen assay that is inexpensive, easy-to-perform, and can be carried out in low-resource settings. Since antiretroviral therapies are becoming more accessible in resource-constrained countries, methods to assess HIV-1 viraemia are urgently needed to achieve a high standard of care in HIV-1 management.
Objectives: To adapt our quantitative assay to dried plasma spots (DPS), in order to further simplify this test and make it more accessible to resource-constrained countries.
Study design: DPS from 47 HIV-seropositive, treated or untreated adult individuals and 30 healthy individuals were examined.
Results: A specificity of 100% was observed when p24 antigen was measured using DPS, and no differences of p24 concentration could be seen between DPS and venous plasma. The correlation between DPS and venous plasma p24 was excellent ($R = 0.93$, $CI_{95\%} = 0.88$–0.96, $p < 0.0001$). Similarly, p24 antigen concentrations using DPS were well correlated with RNA viral load ($R = 0.53$, $CI_{95\%} = 0.27$–0.72, $p = 0.0002$).
Conclusions: This quantitative p24 antigen test has similar sensitivity and specificity using DPS and venous plasma, and has the potential to improve health care delivery to HIV-affected individuals in resource-constrained countries.

Keywords: HIV-1; Treatment monitoring; Viral load; p24 antigen; Dried plasma spots; Low-resource setting

1. Introduction

Today's standard of care for the management of HIV infection includes the quantitative assessment of HIV-1 viremia in order to monitor the response to antiretroviral therapy (ART) or to detect viral relapses. This remains a challenge in resource-constrained countries, mainly because a quantitative assay for HIV-1 remains inaccessible for most patients. Quantitative tests such as reverse transcriptase polymerase chain reaction (RT-PCR), nucleic acid sequence-based amplification (NASBA), or branched chain DNA assay (bDNA), are technically demanding and expensive and, thus, rarely available at the district hospital level. Consequently, ART initiation and treatment monitoring are mostly based on immunological or clinical markers, such as CD4 counts or diagnosis of opportunistic infections.

Introduction of new tests often requires adjustments in the local setting. Inexpensive and easy-to-perform tests are required and simplified sampling and transport procedures may also be necessary. In this respect, the use of dried plasma spots (DPS) on filter papers is a convenient and suitable technique that can be used even in remote district hospitals and rural settings (Behets et al., 1992; Sherman et al., 2005; Stevens et al., 1992).

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Our group has previously developed an ultrasensitive p24 antigen assay that is inexpensive, easy-to-perform, and based on use of venous plasma (Schupbach, 2003). This test does not require large and expensive equipment, which makes it suitable for smaller laboratories. Many clinical studies have been done with this assay using samples from patients with subtype B infections (Mascolini, 2002; Respess et al., 2003, 2005; Sterling et al., 2002). The assay has also been evaluated on non-B subtypes (Burgisser et al., 2000; Ribas et al., 2003) and has been introduced successfully in different African and Asian countries (de Baets et al., 2005; Sutthent et al., 2003; Tehe et al., submitted for publication). Recently, de Baets et al. (2005) described the use of DPS for diagnosis of pediatric infection in Central Africa and reported a 100% specificity and sensitivity for the p24 test. Our goal was to develop a *quantitative* test based on DPS to monitor HIV patients including children under ART. We are herewith reporting the adaptation of the p24 antigen ELISA to DPS in an effort to develop a simple, low-tech, quality-assured method that allows for standard-of-care management of HIV-1 infected patients in remote areas with no direct access to laboratories.

2. Methods

Forty-seven EDTA-anticoagulated venous blood samples were obtained from 42 HIV-1-seropositive, treated or untreated adult individuals. Aliquots of 80 μl plasma were applied to specimen collection papers (Single-part Generic Card, S&S #903, Schleicher & Schuell, Basel, Switzerland) and left to dry overnight at room temperature. These filters were stored until use at 4 °C or −20 °C in small plastic bags containing a desiccant pack (Schleicher & Schuell). Round disks of 16 mm of diameter were cut out, transferred into the wells of 24-well tissue culture plates (NUNC, Wiesbaden, Germany), sealed with a plastic cover sheet, and eluted for 8 h at room temperature into 0.8 ml of an elution buffer containing 1 part H_2O, 1 part SNCR virus lysis buffer (Schupbach et al., 2003), and 9 parts 0.5% Triton X-100. The eluate was denatured for 5 min on a dry-heat block preheated to 100 °C (Techne, Cambridge, UK) and 250 μl/well were then applied to the Perkin-Elmer HIV-1 Core Profile ELISA plate and processed as previously described (Schupbach, 2003; Schupbach et al., 2003) with three modifications to maximize the reaction: (i) the eluated DPS samples were incubated overnight at 37 °C on the ELISA plate, (ii) the detector antibody was incubated for 75 min at 37 °C, and (iii) the horseradish peroxidase was diluted 1:25 in its diluent (for details of procedure see also http://www.nzr.unizh.ch/hivproteinload/index.html). For a few experiments, a different substrate, TMB SureBlue Reserve (KPL, Gaithersburg) was used to improve sensitivity. The p24 concentrations were determined retrospectively and in batches, whereas the viral load and CD4 counts were determined prospectively by routine procedures (Friedl et al., 2001).

3. Results

Thirty HIV-negative individuals as well as 42 known HIV-infected patients were recruited for this evaluation. These patients were infected with HIV-1 subtype B, with the exception of one HIV-1 subtype C and one HIV-1 subtype CRF01_AE infection. Viral loads and CD4 counts were assessed prospectively and are shown in Table 1, together with the characteristics of the individuals. None of the HIV-negative individuals yielded a result above the cut-off of the assay, revealing a specificity of 100% for the dried plasma spots (DPS) p24 assay. For the HIV-1 infected patients, concentrations of p24 in DPS were slightly higher than in plasma (Wilcoxon rank test, $p = 0.027$, $N = 45$). An excellent correlation was obtained for the comparison of DPS p24 and plasma p24 ($R = 0.93$, $CI_{95\%} = 0.88–0.96$, $p < 0.0001$; Fig. 1A).

We further compared the DPS p24 results with those obtained for HIV-1 RNA (Fig. 1B). A significant positive correlation was seen ($R = 0.53$, $CI_{95\%} = 0.27–0.72$, $p = 0.0002$), and similar results were obtained when plasma p24 was correlated with HIV-1 RNA ($R = 0.56$, $CI_{95\%} = 0.32–0.74$, $p < 0.0001$, data not shown). The sensitivity of the p24 test with plasma or DPS was similar with 42/47 (92%) and 41/47 (90%), respectively. The few samples that were undetectable using plasma or DPS were re-tested using TMB SureBlue Reserve (KPL) as substrate, and were then mostly measurable (45/47), conveying a sensitivity of 96% for plasma as well as for DPS p24 antigen. The same two patients were undetectable in both p24 procedures (Fig. 1B, open diamonds), notably, one of them had a sustained viral suppression with occasional blips and an actual viral load of 382 copies/ml. The other patient started therapy during primary infection and experienced a very rapid decrease of HIV RNA load.

Table 1
Characteristics of the HIV-infected patients ($N = 47$, from 42 subjects)

	Median	Intraquartile range (IQR)	Minimum	Maximum
Age (years)	42	47.5–32.5	19	73
HIV RNA copy numbers (copies/ml)	55200	129000–16800	382	480500
Plasma p24 Ag (fg/ml)	8200	30000–2900	100	581500
CD4 T-cell counts (μl^{-1})	266	413–184	3	1031