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Measurement of HIV-1 p24 Antigen by Signal-amplification-boosted ELISA of Heat-denatured Plasma is a Simple and Inexpensive Alternative to Tests for Viral RNA

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Abstract

Assessment of HIV-1 RNA concentration is widely used for monitoring antiretroviral therapies. Tests are, however, expensive and require technically advanced equipment and highly trained personnel. Increasing availability of antiretroviral treatment in resource-poor settings calls for simple and inexpensive virus tests.

HIV-1 p24 antigen tests were frequently used before the availability of nucleic acid tests (NAT). Two simple modifications, heat-mediated destruction of test-interfering antibodies and increased sensitivity achieved by signal amplification, have shaped the p24 antigen test into a tool that rivals NAT. This improved p24 antigen test, for which all reagents are available from Perkin Elmer Life Sciences, was evaluated in clinical studies in comparison with the most sensitive PCR methods available at a given time.

In a prospective study over 4 years, HIV-1 infection among 859 samples from 307 infants born to HIV-positive mothers in Switzerland was detected as sensitively by p24 antigen assay as by PCR for viral DNA or RNA: 100% sensitivity of all methods after 10 days of age; 99.2% diagnostic specificity of p24 after neutralization (RNA, 98.6%). A study conducted in Dar es Salaam (Tanzania) found 123 of 125 samples from 76 PCR-positive infants positive for p24 antigen (sensitivity = 98.7%).

In 169 infected Swiss adults with a median CD4+ T-cell count of 140 cells/µl followed for a median of 2.7 years, p24 at baseline correlated as well as or better than HIV-1 RNA with the ensuing CD4+ T-lymphocyte decline and was independently predictive of progression to clinical AIDS (P = 0.043) and survival (P = 0.032). RNA predicted AIDS (P < 0.005), but not survival (P = 0.19). Another study of first-visit samples from 496 mostly black IVDU in the U.S. with a median CD4+ count of 518 cells/µl showed equally strong prediction of progression to clinical AIDS for p24 antigen, HIV-1 RNA, and CD4+ T-lymphocyte concentrations at baseline.

Treatment-associated changes in p24 and RNA levels in adults and children correlated well in three Swiss studies. The half-life of p24 antigen in the first phase of effective treatment was 1.6 ± 0.4 days (RNA, 1.7 ± 0.8). A second, slower decay phase

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had a half-life of 42 ± 16 days. One study suggested that a strategy involving a somewhat more frequent testing for p24 antigen permitted detection of viral failures significantly earlier than tests for HIV-1 RNA conducted at 3-month intervals, while at the same time significantly saving on costs.
Experience from three studies indicates that the p24 antigen test recognizes viruses of subtypes A - G and O, as well as some recombinant isolates, but leaves open the possibility that some non-B p24 antigens may be suboptimally detected.
This improved p24 antigen test provides diagnosis of pediatric HIV infection, prediction of prognosis and treatment monitoring in quality comparable to tests for HIV-1 RNA, but at much lower costs. There is no problem with sample instability and no need for cumbersome nucleic acid extraction. The test is validated for subtype B, but requires further studies for non-B subtypes.

Key words

HIV. Epidemiology. Africa.

Introduction

Viral load determination has become indispensable for the management of HIV-1-infected patients, with concentration of viral RNA in plasma being predictive of CD4+ T-cell decline, clinical progression and survival[1-5]. Consequently, the proportion of patients who achieve and maintain undetectable viremia has become a major endpoint parameter for clinical evaluation of antiretroviral treatment regimens and for monitoring of treatment in individual patients[6-8].

Antiretroviral therapy is now becoming increasingly available to patients living with HIV/AIDS in many developing countries, due to significant reductions in ARV drug prices. There is, thus, a rapidly increasing need for inexpensive tests capable of assessing the need for antiretroviral therapy in a given patient and of monitoring the effect of such treatment. It is estimated that antiretroviral treatment costs will be in the order of US$ 300 per patient per year. The high costs of the above-described tests, which currently amount to about US$ 100 per test, and the fact that at least two such tests, if not four, will have to be performed every year, seem to preclude their use in the many less affluent countries and societies. In addition, these molecular-based tests require technically advanced and expensive facilities and equipment, as well as highly trained laboratory personnel. It is thus difficult to imagine that these tests could be sensibly used for monitoring of antiretroviral treatment in developing countries. An inexpensive method for measuring the virus load could greatly improve the diagnosis and treatment of HIV infection worldwide.

HIV-1 p24 antigen as an alternative marker of virus expression

Most mechanisms of viral pathogenesis involve viral proteins rather than viral RNA as the direct or indirect mediators of disease. Therefore, there is no rational basis for the assumption that the concentration of viral proteins like the p24 antigen might be less good a marker of disease activity than the particle-associated viral RNA. As a matter of fact, some early studies investigating patients soon after seroconversion have reported that detectability of p24 antigen was a stronger predictor of progression to AIDS than was HIV-1 RNA concentration [9, 10], although all studies performed around this time consistently showed less frequent detection of p24 antigen than of HIV-1 RNA, thus demonstrating a true problem of sensitivity of conventional antigen test procedures[9-12].

P24 and particle-associated HIV-1 RNA are closely related to each other. They are both derived from unspliced viral mRNA, and p24 is a component of the viral protein precursors $Pr160^{gag\text{-}pol}$ and $Pr55^{gag}$, thus being stoechiometrically linked with another precursor component, the nucleocapsid p9, which is directly involved in encapsidation of viral RNA into the particles. P24 is an important structural component of the retroviral particle and estimated to be present at 2000-4000 molecules in each virion[13]. In contrast to HIV-1 RNA, p24 antigen may, however, also be found outside infected cells or particles, as demonstrated by ultracentrifugation experiments (Fig. 1, panel A), either as a soluble protein in plasma, as a protein bound in immune complexes, or as a component of a subviral particle. As a consequence, HIV-1 RNA and p24 antigen, despite being closely interrelated, may origi-

nate from sources, which are common in part, but not identical (Fig. 1, panel B).

Principle and problems of antigen detection

The test principle consists of binding the p24 antigen present in a sample to p24-specific, monoclonal or polyclonal "capture" antibodies which are coated onto a solid support. Unbound sample components are washed away and bound antigen is reacted with another p24-specific "tracer" antibody to which is conjugated an enzyme (horse radish peroxidase or alkaline phosphatase) capable of signal generation when combined with a suitable substrate (Fig. 2A). For confirmation of a reactive diagnostic result, the sample must be subjected to a neutralization assay. This means that the antigen test is repeated in the presence of HIV-specific antibodies. These bind the antigen in immune complexes, thus preventing its detection in the test (Fig. 2B).

This detection system is frequently faced with three problems. One is the presence of p24-specific antibodies, which, as in the neutralization assay (Fig. 2B), immune-complex the antigen, thus causing under-detection or false negative results[14-16]. This problem is exemplified in the course of early HIV-infection during which the increase of antibody concentrations in serum or plasma leads to immune complexation of the antigen and a rapid decrease of its detectability. This may lead to a situation in which Western Blots may still be inconclusive while the antigen test has returned to negative. A second problem is the presence of immunoglobulin-specific, rheumatoid factor-like antibodies, which may bridge the capture and the tracer antibodies of an antigen test and thus cause over-detection or false positive results (Fig. 2C). This type of problem may be present when, in the neutralization test, the addition of HIV-specific antibodies to the test sample does not result in a higher degree of signal reduction than does the addition of antibodies from an HIV-negative control. A third problem is the low sensitivity of the test compared to nucleic acid-based methods[17].

How to improve p24 antigen tests

Improvements introduced into testing were primarily aimed at improving detection of immune-complexed antigen. Acidification or base treatment leads to a significant, though incomplete, release of antigen, thus increasing the proportion of antigen-positives significantly[18]. Experience shows, however, that a considerable part of antigen cannot be freed from complexation or re-associates again when the pH of the sample is neutralized in order to allow binding of the antigen to the capture antibody. In addition, these treatments will release rheumatoid factors from preformed immunoglobulin/anti-immunoglobulin complexes, thus aggravating the problem of over-detection or false positivity[19]. The combination of these two effects, whose extent in a given sample cannot be predicted, prevents an accurate measurement of the true concentration of p24 antigen in a sample.

Functional elimination of interfering antibodies by heat denaturation

During the past few years, significant progress in antigen testing has been made, however. Interference by antibodies (problems 1 and 2) can be eliminated by heat-mediated destruction of the 3D structure of antibodies. Boiling the diluted sample for 5 min abolishes all antigen-binding by antibod-

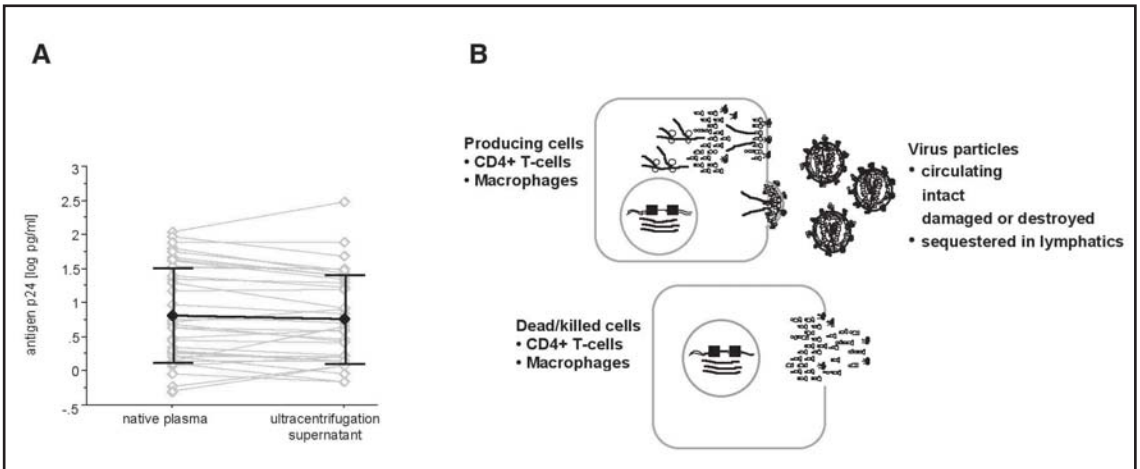

Figure 1. *Panel A. Evidence for presence of p24 outside viral particles. Ultracentrifugation of plasma from patients in the chronic stage of HIV infection, while removing all viral RNA, leaves most of the p24 antigen in the supernatant thus indicating that most of the detectable antigen is not associated with viral particles.*
Panel B. Possible sources of p24 antigen and HIV-1 RNA in plasma. P24 antigen may originate from several sources including the structural protein of intact or defective viral particles present in the sample or released from particles degraded while entangled in the follicular dendritic cell network of the lymphatics. P24 antigen may also be released from HIV producing cells or leak from cells killed either by viral or immune-mediated cytotoxicity. P24 antigen concentration in plasma may therefore be more representative of the total viral load in the body than is the HIV-1 RNA in plasma, which originates exclusively from intact circulating particles.

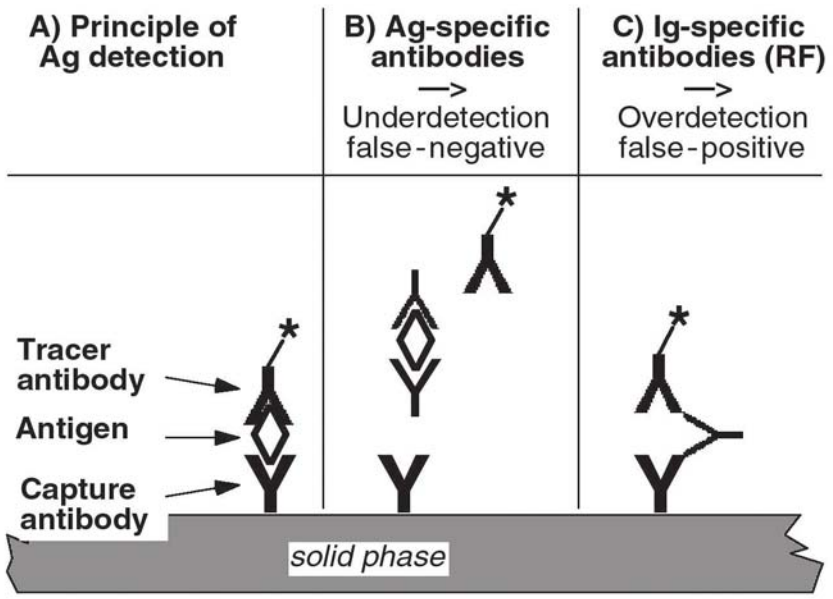

Figure 2. *Principle of antigen testing and interference by antigen-specific or Ig-specific antibodies. For explanation see text. Reprinted with permission from[36].*

ies, but leaves the p24 antigen reactive in tests that feature reagents (mono- or polyclonal antibodies for capturing and tracing) which recognize heat-denatured antigen[20]. This effect has been demonstrated in numerous experiments involving both artificial immune complexes and natural patient samples. Thus, this simple measure permits measurement of a sample's true antigen content (Fig. 3).

The value of this initial heat-denaturation-based procedure was firmly established by a study of children born to HIV-1-infected mothers in Switzerland. In this retrospective study the procedure's specificity in 390 samples from uninfected children born to HIV-positive mothers was 96.9% after initial testing and 100% after neutralization. Diagnostic sensitivity among 125 samples from infected children was, at a detection limit of 2 pg/mL, 96.0% (97% of which neutralizable), compared with 47.7% for regular antigen (76% neutralizable), 96% for PCR for HIV-1 DNA, and 77% for virus culture[21]. The study also found low levels of p24 antigen in 29% of cord blood sera, a postnatal increase to levels that were during the first 6 months of life - i.e., the time of the primary infection - inversely correlated with survival, and persistence of antigenemia in all children thereafter. These findings were in complete agreement with the subsequent demonstration by others that,

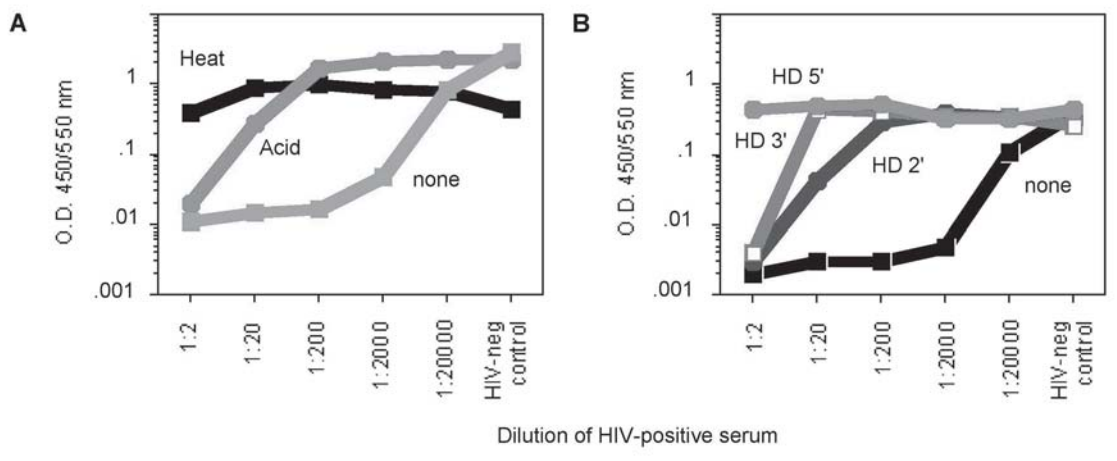

Figure 3. *Effect of immune complex dissociation methods on artificial immune complexes of varying antibody/antigen ratio. Model IC were formed by overnight incubation of HIV-positive serum dilutions admixed to a constant volume of HIV-negative serum containing 100 pg/ml of exogenous HIV antigen. Panel A. Comparison of antigen detected in undenatured, acidified, and heat-denatured samples. Panel B. Effect of heat denaturation time (HD, in minutes) upon detection of immune-complexed antigen. Modified with permission from[20].*

in perinatally infected children, high viral RNA levels at birth and during primary viremia were associated with the early onset of symptoms and rapid disease progression to AIDS and death[22].

Increase of sensitivity by tyramide signal amplification

Despite its high diagnostic sensitivity in pediatric HIV infection, the procedure was still not sufficiently sensitive, as shown by the fact that only 22% of the mothers of these children tested positive[21]. The antigen assay was, therefore, boosted by the simple, commercially available, tyramide signal amplification system. The principle of this signal amplification system is shown in Fig. 4[23]. A comparison of paired serum and plasma samples from 245 adult HIV-1-infected individuals at all stages of chronic infection showed, furthermore, that plasma contains more p24 antigen than serum (Fig. 5). In combination, heat denaturation, use of plasma instead of serum and tyramide signal amplification led to a procedure that had the same diagnostic sensitivity as the Roche Amplicor HIV-1 Monitor® Version 1.0 (Table 1)[24]. The high sensitivity and practical utility of the procedure was also confirmed by others in a study of African children from Tanzania[25]. Finally, use of a commercial software allowing combined kinetic and endpoint evaluation of the ELISA reaction on a broad variety of ELISA readers allowed quantification from about 0.5 pg/ml to more than 6 ng/ml, thus permitting antigen quantification at a single dilution with virtually all clinical samples[26].

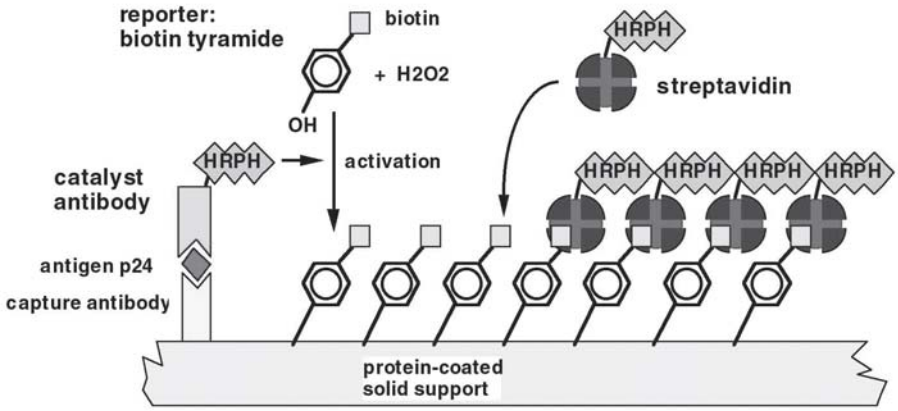

Figure 4. *Principle of tyramide-mediated signal amplification of ELISA[23]. The tracer antibody which is labeled with horseradish peroxidase H (HRPH) is used as a catalyst antibody for the activation of the biotin tyramide reporter molecule. The activated reporter binds to tyrosine residues of any immobilized protein. Added HRPH-labeled streptavidin thus finds a highly increased number of targets thereby generating an enhanced signal. Reprinted with permission from[37].*

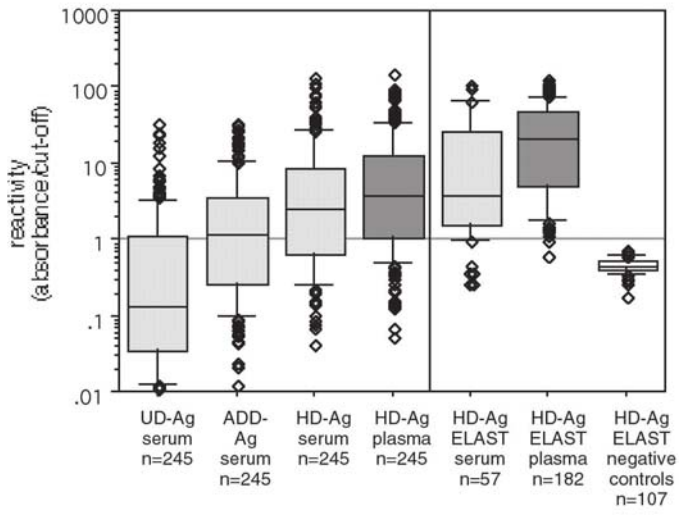

Figure 5. *Overview on the effects achieved by the various measures used to improve antigen detection. The box plot rendition of the reactivity of each sample is a percentile-based analysis, in which the five horizontal lines represent, from bottom to top, the 10th, 25th, 50th, 75th and respectively 90th percentile and outrunners are plotted individually. UD-Ag, undenatured antigen; ADD-Ag, acid-disruption difference antigen; HD-Ag, heat-denatured antigen; HD-Ag ELAST, heat-denatured antigen combined with detection by ELAST® tyramide signal amplification boosted ELISA. Reprinted with permission from[24].*

Table 1. *Virus component detection by signal-amplification-boosted p24 antigen ELISA of heat-denatured plasma and PCR for HIV-1 RNA. Reprinted with permission from*[24]

Classification	p24 antigen ELISA		HIV-1 Monitor Vs. 1.0	
	positive/tested	%	positive/tested	%
by CDC 93 category				
A	71*/74	95.9	46/50	92
B	50*/51	98.0	31/32	96.9
C	57/57	100	35/35	100
by CD4+ cell category				
1 (≥500/µl)	12/14	85.7	6/6	100
2 (200-499/µl)	52*/53	98.1	34/38	89.5
3 (<200/µl)	114*/115	99.1	72/73	98.6
Total	**178/182**	**97.8**	**112/117**	**95.7**

*After subtraction of one reactive sample not confirmed by neutralization

Evaluation of the improved p24 antigen test

Since the development of the amplification-boosted procedure in 1996, several studies were conducted in order to compare the assay with PCR-based methods. For all antigen assays the HIV-1 p24 Core Profile ELISA in combination with the ELAST® ELISA Amplification System was used; both components are now available as a single kit from Perkin Elmer Life Systems. If not otherwise stated, the Amplicor HIV-1 Monitor® Version 1.0 (400 copies/ml) was used for quantification of viral RNA. For diagnostic purposes, qualitative in-house tests for viral DNA or RNA capable of detecting a single copy of HIV-1 DNA respectively, cDNA were used[21,27].

Diagnosis of pediatric HIV-1 infection

A prospective study over four years with real-time analysis of p24, HIV-1 DNA and RNA investigated the diagnostic sensitivity of p24 antigen and PCR-based tests in 232 samples from 61 HIV-1-infected, untreated children born to HIV-positive mothers in Switzerland (Table 2)[28]. All tests were 100% positive above 10 days of age. Below 10 days, p24 was confirmed positive in six of 12 samples. DNA PCR and in-house PCR for viral RNA both missed one of the samples positive for p24. When retested by the HIV-1 Monitor version 1.5 *ultrasensitive* assay with a detection limit of 50 copies/ml, the sample was also negative. The diagnostic specificity of the p24 assay among 643 plasma samples from 246 uninfected children born to HIV-1-positive mothers was 99.2% after neutralization (Table 5). Two (1.4%) of 141 samples tested with the in-house method for viral RNA were false-positive, resulting in a diagnostic specificity of 98.6%. Thus, p24 was equal to RNA regarding diagnostic sensitivity and specificity in pediatric HIV-1 infection.

Diurnal variation of HIV-1 p24 antigen concentration in plasma and precision

Few data are available on the precision of the p24 antigen assay, but they suggest a higher precision than that of the HIV-1 Monitor assay. Diurnal variation of plasma HIV-1 load at four different time points, each during two different days (a Friday and the following Monday), was studied in five HIV-1-infected children with implanted intravascular catheters, after informed consent. The investigations demonstrated that the p24 antigen levels had, with a mean log standard deviation (SD) that amounted to 0.057 (range 0.02 - 0.11), less varia-

Table 2. *Diagnostic sensitivity of HIV-1 detection methods in pediatric samples. Reprinted with permission from*[28]

Age	Antigen neutralized	in-house PCR viral DNA	in-house PCR viral RNA	HIV-1 Monitor viral RNA
<10 days	6/12 (50)*	5/12 (42)	3/7 (43)	not done**
11 days - 3 months	10/10	8/8	7/7	6/6
>3-6 months	19/19	12/12	12/12	9/9
>6 months	191/191	66/66	26/26	120/120
>10 days	220/220 (100)	86/86 (100)	45/45 (100)	135/135 (100)

*Shown are n positive / n tested samples (%)
**The sample positive in the antigen assay but negative by in-house PCR for viral DNA respective RNA was also negative by the ultrasensitive HIV-1 Monitor version 1.5

tion than the HIV-1 RNA concentrations (mean log 0.108; range 0.07 - 0.15)[28]. In another study, six different specimens were tested 3-4 times in an assay; the mean log SD of the antigen test was 0.07 (range 0.03 - 0.12), while it was 0.11 (0.07 - 0.02) for the Roche HIV-1 Monitor assay[29].

Prediction of disease progression

The predictive value of p24 antigen concentration was tested in two different studies. In a first, retrospective study involving 169 chronically-infected adult Swiss patients with a median CD4+ T-lymphocyte count of 140 cells/µl (range 0 - 1500), p24 antigen and HIV-1 RNA concentrations were determined in a single sample collected in 1993-1994 and the predictive value of these markers regarding disease progression was compared. Follow-up data included at least one further CD4+ T-lymphocyte count and assessment of clinical stage, with a median observation period of 2.7 years (range 0.1-4.9). In CD4-adjusted Cox' proportional hazard models, both RNA ($P < 0.005$) and p24 antigen ($P = 0.043$) were significant predictors of progression to AIDS. P24 was superior ($P = 0.032$) to RNA ($P = 0.19$; n.s.) in predicting survival. P24 was also a significant predictor of the CD4+ decline in 'CD4+-adjusted' models and was equivalent or superior to HIV-1 RNA, depending on the group analyzed and the statistical test employed[30]. The prognostic value of p24 antigen was confirmed in a second study, which involved first-visit samples from 494 mostly black IVDU from Baltimore, Maryland. This cohort had a median initial CD4+ lymphocyte count of 518/µl; 90 of the patients (18%) progressed to AIDS within 5 years. P24 antigen was strongly correlated with both HIV-1 RNA ($r = 0.55$; $P < 0.0001$) and CD4+ lymphocytes ($r = -.34$; $P < 0.0001$). P24 level >5 pg/ml predicted disease progression comparable to cutoffs of <350 CD4+ lymphocytes/mm^3 and >30,000 copies/ml HIV-1 RNA. Heat-denatured p24 antigen thus predicted subsequent clinical disease progression in early-stage HIV-1 infection, and was closely correlated with both CD4+ lymphocyte and HIV-1 RNA level[31].

Antiretroviral treatment monitoring and detection of treatment failures

The suitability of p24 antigen for antiretroviral treatment (ART) monitoring was investigated in both adult and pediatric infection of patients in Switzerland. In a study of 23 adult patients with advanced disease who received a new, indinavir-containing treatment regimen, p24 antigen was detected as sensitively as viral RNA, namely in 75.6% of the samples (RNA, 73.6%). Antigen and RNA levels in 79 samples positive for both markers correlated with $R = 0.714$ ($P < 0.0001$). This correlation was similar to that found in a different study in which HIV-1 RNA levels were determined in parallel by two different methods, namely the Amplicor HIV-1 Monitor® and the NucliSens® HIV-1 RNA Quantitative Test[32]. Average changes in levels of p24 antigen and RNA at eight time-points correlated with $R = 0.982$ ($P < 0.0001$; Fig. 6). In individual patients, the two parameters behaved similarly and, in certain cases, virtually identically[33]. Similar results were found in a prospective study of 25 children with a total of 230 analyzed samples in Switzerland. Here, the correlation of RNA and p24 antigen was $R = 0.658$ ($P < 0.0001$). In most instances the treatment-induced changes were more pronounced for HIV-1 RNA than for p24. P24 levels showed significantly less variation than HIV-1 RNA.[28].

In a recently published paper, we investigated 34 Swiss patients who were enrolled during 1997 into two treatment studies in which they were prospectively tested for viral RNA by the Roche HIV-1 Monitor® version 1.0 and p24 antigen[24]. The data were evaluated regarding the response of these markers to antiretroviral treatment and timely detection of treatment failures. We found that p24 antigen was detectable in 75.8% of 178 samples and HIV RNA in 73.9% of 138 samples. The half-life of p24 antigen in the first phase of effective treatment was 1.6 ± 0.4 days (RNA, 1.7 ± 0.8). A second, slower decay phase had a half-life of 42 ± 16 days. Treatment failure, as defined by RNA concentrations, occurred in 14 patients. Secondary treatment failures with RNA rebounds from undetectable levels to less than 10^3 copies/ml in two patients with an undetectable viral load and 10^3 HIV RNA copies/ml, respectively, at baseline were not confirmably detected by p24 antigen. The two failures carried a low risk for secondary resistance mutations and were, as demonstrated by retesting with a still more sensitive p24 antigen assay, in principle detectable. The other 12 failures were detected, on average, 29 days earlier by p24 antigen than by RNA ($P = 0.020$), owing to slightly more frequent testing for p24 antigen than for RNA (2.7 versus 2.4 tests until detection of treatment failure). Average costs of p24 antigen testing up to failure were only 20.5% of those of RNA ($P < 0.0001$).

In a further study we evaluated p24 antigen in patients receiving successful long-term ART (Schupbach, et al., submitted). HIV-1 RNA and p24 antigen were prospectively measured in 329 samples from 55 patients whose HIV-1 RNA had been reduced to below 50 copies/ml and who continued to receive therapy. HIV-1 RNA in PBMC was determined retrospectively. Uni- and multivariate linear regression analysis was used to correlate CD4+ counts and their changes over time with the counts or changes of total lymphocytes, CD3+ T-lymphocytes, CD8+ T-lymphocytes, HIV-1 RNA, and p24. During a median follow-up of 504 days, CD4+ counts increased by a median of 62 cells/year. Concentrations of lymphocytes and their subsets strongly influenced the CD4+ count. P24 was a significant, and among viral markers dominating, independent inverse correlate of both the CD4+ count in a sample ($P = 0.013$) and its annual change in a patient ($P < 0.0001$). P24 retained similar significance even among 48 individuals who never had a viral rebound to higher than 400 copies/ml.

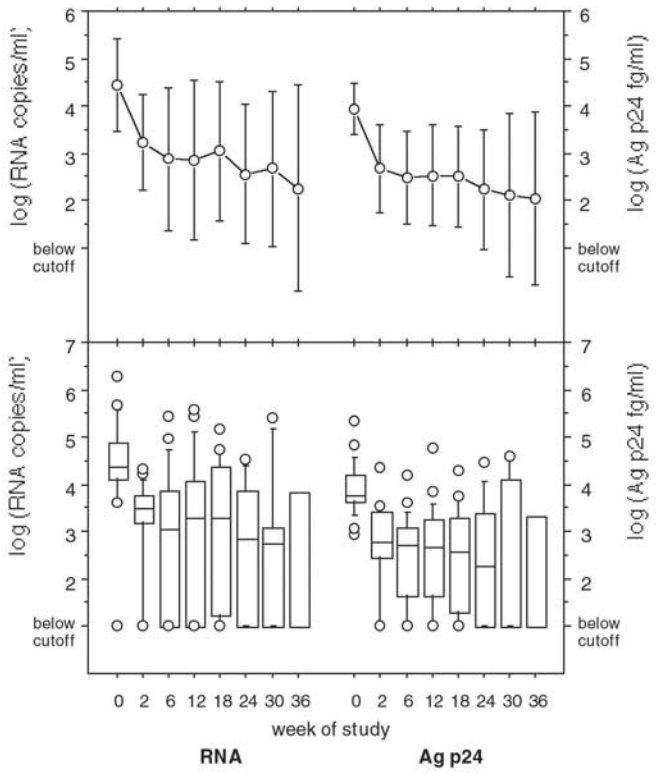

Figure 6. *Treatment-induced changes in concentrations of HIV-1 RNA and p24 antigen. Shown are logarithmized mean values ± 1 standard deviation (upper panel) and box plots in which the horizontal lines represent, from top to bottom, the 10th, 25th, 50th, 75th and 90th percentile, respectively (lower panel). Reprinted with permission from[33].*

Detection of non-B subtype viruses

The results described above indicate that p24 is comparable to HIV-1 RNA when used for diagnosis of pediatric HIV-1 infection, as a marker of disease progression, or for treatment monitoring in Switzerland or the U.S. where subtype B samples are prevalent. With regard to the use of this test in developing countries, in particular Africa, it is important to assess the suitability for non-B subtypes. Only limited data regarding this issue are currently available. Lyamuya, et al. found a high diagnostic sensitivity in diagnosing pediatric HIV-1 infection in Dar es Salaam, Tanzania. Altogether, 123 of 125 samples from 76 PCR-positive infants were positive for p24 antigen (sensitivity = 98.7%). HIV-1 p24 antigen was found in all 18 samples collected at 1-8 weeks, in 35 of 36 samples collected at 9-26 weeks, in all 40 samples collected at 27-52 weeks, and in 30 of 31 samples collected 52 weeks after birth [25]. The sensitivity of the assay was also assessed in a Swiss study of 103 individuals likely to be infected by non-B subtypes[34]. The tests assessed included three RNA-based assays including the Amplicor HIV-1 Monitor 1.5, the Quantiplex version 2.0 (bDNA), the NucliSens (NASBA), an ultrasensitive reverse transcriptase assay called PERT assay[35], and the improved p24 antigen assay. Subtyping was based on sequencing in the *env* gene. P24 was more sensitive than NucliSens or Quantiplex, but less sensitive than Amplicor or PERT assay (Table 3). A more detailed, quantitative comparison showed that two samples with a HIV-1 RNA concentration above 10,000 copies/ml (one subtype A and one subtype C) were negative for p24 antigen. Other samples of these subtypes were, however, well recognized, even some in which HIV-1 RNA was not detectable or below the limit of quantification (400 copies/ml). In particular, the p24 antigen assay was also positive in one subtype O sample that was negative by all assays for HIV-1 RNA, but positive by the PERT assay. Good detection of subtypes A - F was also reported by others[29]. These data suggest that the p24 antigen assay is not *per se* inferior to tests for HIV-1 RNA regarding recognition of different subtypes. However, this issue needs to be studied more extensively before the test is routinely used in non-B areas, and adjustments regarding the capture or tracer antibodies of the kit may prove necessary.

Sample handling and physical stability of p24 antigen

In an attempt to further strengthen the evidence for a predictive value of p24 antigen, we recently conducted a study involving serum samples collected between 1989 and 1990 from 547 patients of all disease stages treated at the Zurich University Hospital (J. Schüpbach, B. Ledergerber, et al., manuscript in preparation). The study intend-

Table 3. *Sensitivity of five different assays for the detection of HIV-1 in plasma of 83 patients. Reprinted with permission from*[34]

Subtype	No. of samples[a]	No. of samples testing positive				
		Amplicor 1.5	Quantiplex 2.0	NucliSens	PERT	Boosted p24 antigen ELISA
A	25	19	10	10	16	15
B	15	9	2	7	5	5
C	8	6	3	3	4	3
D	3	2	1	2	1	0
E	10	6	3	4	5	3
F	1	1	0	0	1	0
G	1	1	1	0	1	1
Recombinant	10	6	5	3	6	7
O	1	0	0	0	1	1
Unknown	9	5	1	1	3	3
Total	**83**	**55** (66.3%)	**26** (31.3%)	**30** (36.1%)	**43** (51.8%)	**38** (45.8%)
Untreated (n = 25)[b]		84.0%	56.0%	60.0%	84.0%	68.0%
Treated (n = 58)[b]		56.9%	20.7%	25.9%	37.9%	36.2%

[a]Includes only samples for which results by all five methods are available. Very similar results were obtained when samples with missing data were included (Amplicor (Roche), 66.3%; Quantiplex (Chiron), 33.7%; NucliSens (Organon Teknika), 37.5%; PERT assay, 50.0%; p24 Ag EIA, 43.1%)
[b]Antiretroviral treatment

ed to directly compare the predictive values of HIV-1 p24 antigen and HIV-1 RNA. Unfortunately, HIV-1 RNA was found to be degraded in the majority of samples, and the study had to be restricted to assessment of p24 antigen alone. Of the 547 samples, 92.5% had a p24 antigen concentration above the cut-off; these samples exhibited the same concentration distribution as previously noted in another study[30]. These data indicate that p24 antigen is much more stable than viral RNA. In accordance with this, there is no need for special "plasma preparation tubes", expensive individual express delivery, and -70°C freezers. Samples may be kept for several days at 4°C before testing. Preliminary assessment of the effect of freezing/thawing cycles has indicated that one such cycle leads to about 3% loss of p24 antigen, with no further change after the 3rd cycle (J. Schüpbach, unpublished).

Costs

In the absence of a kit that contained all necessary ingredients for sample preparation, ELISA, and signal amplification, it was difficult to assess the price of this test. Previous comparisons based on reimbursement by health insurances in Switzerland arrived at a price of sFr. 50 (US$ 30) for the antigen test and sFr. 275 (US$ 167) for a HIV-1 Monitor assay. A study performed in the U.S. quoted US$ 8 for the antigen assay and US$ 75 for the HIV-1 Monitor assay including reagents and work[29]. Based on these data, the costs of the p24 assay can be expected to amount to 10 - 20% of those of a HIV-1 RNA assay.

Conclusions

The studies briefly reviewed here indicate that the p24 antigen, if assessed by proper methodology, is comparable to viral RNA with regard to sensitivity and specificity, correlation with counts of CD4+ T-cells and their changes, prediction of CD4+ T-cell decline and progression to AIDS or death, and monitoring of antiretroviral treatment. Unlike HIV-1 RNA measurement, this simple, considerably less expensive and easily automatable procedure does not require cumbersome sample transport and pretreatment procedures. Further studies on p24 antigen are highly warranted; in particular, they should aim at validating the test for non-B subtypes.

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