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Short communication

Evaluation of four HIV antigen tests

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Abstract

A direct comparison of different HIV antigen assays is very helpful in making an informed choice, not only for the testing laboratories but also for healthcare workers in the developing world who are looking for reliable and inexpensive tests/methods in the follow-up of their treated patients. As a follow-up to the study published previously [Fransen K., Martens G., Stynen D., Goris A., Nys P., Nkengasong J., Heyndrickx L., Janssens W., van der Groen G., 1997. J. Med. Virol. 53, 31–35] where only two tests have been compared, four different commercial methods for HIV antigen determination in plasma and supernatant of cell cultures have now been evaluated on a limited sample size (88): COULTER™ HIV-1 p24 Antigen Assay (Coulter), (Test 1) INNOTEST HIV Antigen mAb (Innogenetics) (Test 2), Genetic Systems™ HIV-1 Ag EIA (Sanofi-Pasteur [1]) (Test 3) and VIDAS HIV P24 II (bioMérieux) (Test 4). Of the four tests used in this study, Test 2 was by far the most sensitive test. In a population of 88 follow-up samples from 35 different patients representing all stages of infection, the test detected confirmed p24 antigen at least once in 85.7% (30/35) of these patients, versus Test 3 in 74.3% (26/35), Test 4 in 71.4% (25/35), and Test 1 in 48.6% (17/35) of the patients. Test 2 detected confirmed p24 antigen in 84.9% of the follow-up samples, followed by Test 4 (65.9%), Test 3 (64.8%) and Test 1 (39.8%). Finally, Test 2 also proved best for detecting genetically diverse isolates.

Keywords: p24 antigen; HIV; SIV; Antigen detection

1. Material and methods

1.1. Patient samples

From a previous study, 88 follow-up samples were retained from 35 different patients with proven HIV-1 infection, with samples drawn at diverse stages of the HIV disease process (eight acquired immunodeficiency syndrome (AIDS), five persistent general lymphadenopathy (PGL),

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[1] The company Sanofi-Pasteur is now referred to as Biorad.

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one AIDS-related complex (ARC), 20 asymptomatics, and one unknown.

1.2. Culture supernatants

Similarly prepared supernatants (aliquots) from 18 different HIV types (eight group M HIV-1 strains from different subtypes, four HIV-1 group O, four HIV-2, and two SIV cpz strains) were used. Threefold dilution series of the culture supernatants can therefore be compared between four different commercial tests. The subtypes have been determined previously according to different methods as described previously (Fransen et al., 1997).

At least one of the following tests was used to confirm HIV infection: co-culture with phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMCs) of healthy HIV seronegative human donors (Nkengasong et al., 1995), Western blot HIV Blot 2,2 (Genelabs Diagnostic, Geneva, Switzerland) or New Lav Blot I and II (Sanofi Diagnostics Pasteur), INNO-LIA HIV-1/HIV-2 Ab (Innogenetics, Zwijndrecht, Belgium) or HIV ab (rDNA) p24 ELISA (Abbott, No. Chicago, IL).

1.3. Assays

Two tests were used in a previous, more extensive evaluation: Test 1 (reference test): COULTER™ HIV-1 p24 Antigen Assay including the COULTER HIV-1 p24 Antigen Neutralisation Kit (Coulter), is an EIA for the detection of p24 antigen of HIV virus type 1 (HIV-1) in plasma, serum or tissue culture media. Limit of detection is not mentioned in the instructions.

Test 2: INNOTEST HIV Antigen mAb (Innogenetics) including the INNOTEST HIV Antigen mAb Neutralization Reagents (Innogenetics), is an EIA for the detection and quantification of p24 core antigens of HIV-1, HIV-1 group O and HIV-2 in human serum, plasma, or cell culture supernatant. Limit of detection according to the instructions is 10 pg p24 core antigen/ml.

Two tests were added in this evaluation: Test 3: Genetic Systems™ HIV-1 Ag EIA, including the Genetic Systems HIV-1 Ag EIA Confirmation procedure (Sanofi-Pasteur), an EIA for the detection of uncomplexed HIV-1 p24 antigen in human serum, plasma, and cell culture supernatant. Limit of detection according to the instructions is 8 pg of HIV-1 antigen/ml.

Test 4: VIDAS HIV P24 II, including VIDAS HIV P24 Confirmation (bioMérieux), is an automated enzyme-linked fluorescent immunoassay for the determination of p24 antigen of HIV-1 in human serum or plasma. Limit of detection according to the instructions is 11.25 pg of HIV antigen/ml or 3.1 pg of p24 antigen/ml. An adapted protocol for the use of the VIDAS HIVP24 II with culture supernatant was necessary to perform the evaluation on the culture supernatants.

The tests were carried out according to the manufacturer's instructions. All positive samples were treated with the respective confirmation kits according the manufacturers instructions.

2. Results

All the samples were analysed in parallel using the four tests. Tests 3 and 4 were performed later in time on aliquots kept at -80°C. To ascertain whether any deterioration of the samples had occurred, 10 weak positive samples in Test 2 were retested and again remained positive.

As seen in Table 1, 36 samples on a total of 88 follow-up samples of HIV infected individuals were initially reactive with Test 1, 75 with Test 2, 59 with Test 3 and 56 ($+5$ equivocal) with Test 4. After repeat testing and/or neutralisation, 35 samples of the 36 samples remained positive with Test 1, 73 on 75 with Test 2, 57 on 59 with Test 3 and 58 on 61 ($56 = 5$) with Test 4. Two initially positive samples in Test 2 were not tested in the neutralisation. Only two of the four equivocal samples in Test 4 were confirmed as positive. Antigen was detected in 17 (48.6%), 30 (85.7%), 26 (74.3%) and 25 (71.4%) of 35 HIV-infected patients with antigen Tests 1, 2, 3, and 4, respectively. All samples ($n = 17$) positive with Test 1 were also positive with the other three. A total of 24 different patient samples were detected with both Tests 3 and 4; one sample was not detected with Test 3 but was positive with Test 4, while

two patient samples positive with Test 3 were not detected with Test 4. All samples positive with either Test 3 and 4 were also positive with Test 2.

Table 2 compares the sensitivity of the three tests using Test 1 as a reference (for comparison of the data with previous results (Fransen et al., 1997), using supernatant from cultures of different HIV types and subtypes. The sensitivity factor was determined as the ratio of the HIV antigen titer (reciprocal of the highest dilution for which the test is still positive) of the test under evaluation divided by the titer of the reference test. The reference test was only more sensitive (with one dilution) than the most sensitive Test 2 for one single isolate 6 (CA 4). For most of the other isolates belonging to HIV-1 group M, HIV-1 group O, and SIV cpz, the sensitivity of Test 2 was higher than the sensitivity of the other three tests. Only isolate 5 (CA 10), 7 and isolate 8 (VI 991) scored better in Test 3 compared to Test 2. The sensitivity for HIV-2 isolates was far the best with Test 2. No other proved more sensitive than Test 2.

3. Discussion

The advantage in the use of p24 antigen detection lies in early detection of an HIV infection. The high (best) sensitivity of Test 2 was demonstrated previously by Louwagie et al. (1996) by comparing test results of five different antigen tests on 28 seroconversion panels. Although the trend in most laboratories is to switch to fourth generation tests, which is a combined antibody–antigen test, a single antigen test still has a higher sensitivity in detecting antigen compared to a combined test (Couroucé et al. 1999). The importance of a specific and sensitive antigen test for monitoring of antiviral therapy has diminished in the developed world, with the routine determination of the plasma viral load, but it could become more important in the follow-up of patients where resources are scarce (developing world) and therapy is in its infancy. Studies by Schüpbach et al. (1996), Böni et al. (1997) and Nadal et al. (1999) indicate that p24 antigen, if assessed by proper methodology, is comparable to viral RNA with respect to sensitivity and specificity in monitoring of disease progression. Unlike RNA measurements, the simple, inexpensive and easily automatable p24 antigen detection procedure does not require cumbersome sample transport and pre-treatment procedures. Compared to viral load assays, the average costs are cheaper by a factor 20 times.

p24 detection may also be of interest as a simple and inexpensive predictive marker of disease progression. Both RNA and p24 levels are significant predictors of progression to AIDS. Measurements of p24 levels was even shown to be superior to measurements of RNA in the survival model. p24 level is a significant predictor of CD4+ cell decline in models adjusted for CD4+ cell counts, and is superior (CD4+ <200 μl) or equivalent (CD4+ >200 μl) to measurement of RNA levels (Ledergerber et al., 2000). The fact that HIV antigen detection still plays an important tool in virus culture, has been discussed in a previous article. Briefly, it is still used for determination of endpoint titers of infectious HIV and in

Table 1
Sensitivity of four antigen tests on HIV antibody positive follow-up samples

	Test 1[b]	Test 2[c]	Test 3[d]	Test 4[e]
Follow-up samples $n=88$				
Initially positive	36 (40.9%)	75 (85.2%)	59 (67.0%)	56 (63.6%)
After neutralisation/confirmation	35 (39.8%)	73[a] (84.9%)	57 (64.8%)	58 (65.9%)

[a] $n=86$. Two initially positive samples in Test 2 could not be retested in neutralisation.
[b] Test 1, COULTER™ HIV-1 p24 Antigen Assay (Coulter).
[c] Test 2, INNOTEST HIV Antigen mAb (Innogenetics).
[d] Test 3, Genetic Systems HIV Ag EIA (Sanofi-Pasteur).
[e] Test 4, VIDAS HIV-p24 (bioMérieux).

Table 2
Difference in sensitivity of four antigen tests on culture supernatants of different types and subtypes of HIV and SIV

Type	Isolate	Name	Subtype Env/Gag	Sensitivity factor[a]		
				Test 2[b]	Test 3[c]	Test 4[d]
	1	CA 1	A/A	9	9	9
	2	CA 5	B/B	3	1	3
	3	VI 313	C/A	3	1	0.3
	4	VI 824	D/D	1	1	0.004
	5	CA 10	A/E	3	27	1
HIV-1	6	CA 4	D/F	0.3	0.3	1
	7	VI 1197	A/G	9	3	3
	8	VI 991	H/H	3	9	0.004
	9	ANT-70	O/O	9	0.004	1
	10	VI 686	O/O	3	0.1	0.3
	11	CA 9	O/O	3	1	1
	12	MVP 5180	O/O	9	3	3
HIV-2	13	LAV-2	−/A	729	0.1	3
	14	VI 53	−/A	2187	1	27
	15	VI 884	−/A	729	0.1	3
	16	VI 1415	−/A	19683	1	81
SIV cpz	17	SIVcpz-ant	-	9	0.3	3
	18	SIVcpz-gab	-	27	27	9

[a] The sensitivity factor was determined as the ratio of the HIV Ag titer (reciprocal of the highest dilution for which the test is positive) of the test under evaluation divided by the titer of the reference test. The reference test is the COULTER™ HIV-1 p24 Antigen Assay (Coulter).
[b] Test 2, INNOTEST HIV Antigen mAb (Innogenetics).
[c] Test 3, Genetic Systems HIV Ag EIA (Sanofi-Pasteur).
[d] Test 4, VIDAS HIV-p24 (bioMérieux).

determining infectivity in the different antibody neutralisation tests (Beirnaert et al., 1998). In the VIDAS HIV P24 II test, plasma and supernatant samples cannot be tested in the same run. An adapted protocol for supernatant had to be used.

The genetic variability of HIV can be very diverse in the developing world and, therefore, the antigen-capturing test should be able to detect antigens of different types and subtypes. Of the four assays evaluated the INNOTEST HIV Antigen mAb is a good candidate to be evaluated for these purposes.

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