Molecular specificity of two commercial enzyme linked immunosorbent assays for human immunodeficiency virus antigens

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Abstract

Only "fair" agreement has been shown between the Abbott and DuPont enzyme linked immunosorbent assays when used for the detection of human immunodeficiency virus (HIV) antigen in serum samples from asymptomatic HIV antibody positive homosexual men. To investigate the discrepancies between the two ELISA results, further experiments were performed. The rabbit detector antibody solutions of both tests were western blotted and showed that the DuPont test was specific for p24; the Abbott detector antibody had bands for p18, p41-43, gp120 as well as p24. By using dilutions of a known amount of HIV antigen, the Abbott test could detect 20 pg/ml p24; the DuPont test could detect 30 pg/ml p24. The DuPont test was also more sensitive than the Abbott test at detecting a synthetic 104mer peptide of p24. Within the 104mer sequence two regions (294-318, 334-348 amino acids) inhibited the binding of the DuPont detector antibody, but no blocking was observed with the Abbott antibody.

Although the Abbott test was slightly more sensitive at detecting HIV protein than the DuPont test, the major difference between the tests was in the molecular specificity, in that the Abbott test detected proteins other than p24. This may not be important for detecting antigen in cell culture, but it may affect the detection of antigenaemia in patients' sera.

Human immunodeficiency virus (HIV) antigen tests are widely used to monitor patients with HIV infection. HIV antigen can be detected in the serum before antibody seroconversion, but it then disappears in most cases as antibody titres become detectable. Patients who have persistent HIV antigenaemia seem to be at a higher risk of developing AIDS.¹ HIV antigen tests have been recommended as a useful index for starting and monitoring patients on antiviral treatment.²⁻⁵

Two commercially available HIV antigen assays (Abbott HTLV III Antigen test and the DuPont HIV p24 test) are widely used for these assays. We have previously tested asymp-

tomatic HIV antibody positive homosexual men using both tests, and shown only "fair" agreement between the results of the two tests.⁶ In this study we examined the relative sensitivities and molecular specificities of the assays in an attempt to account for the discrepancies between the tests.

Methods

SENSITIVITY AND CALIBRATION OF THE ASSAYS

A series of dilutions were made from a viral lysate containing 200 ng/ml p24 (800 ng/ml total HIV protein) (EI DuPont de Nemours and Co.). The resultant solutions were tested (in duplicate) in both ELISA systems using the manufacturer's protocol. By incorporating each ELISA's own test standards, each assay could also be calibrated against the known antigen titre.

SPECIFICITY OF THE ASSAYS

Western blotting of the rabbit detector antibody
Nitrocellulose strips with pre-electrophoresed HIV proteins (BioRad Laboratories Ltd, Watford) were washed on a side to side rocker for two hours in phosphate buffered saline (PBS) containing 5% bovine serum albumin (BSA). The strips were then incubated overnight with 1.5 ml of detector antibody in 1.5 ml PBS containing 1% BSA and 0.05% Tween 20.

After washing three times in PBS the strips were incubated for two hours at room temperature with a 1 in 250 dilution of anti-rabbit IgG biotin conjugate (Amersham). The strips were rewashed three times in PBS and reincubated for two hours at room temperature in a 1 in 250 dilution of streptavidin-horseradish peroxidase (Amersham). After final washes in PBS the colour was developed using 1 volume of 3 mg/ml 4-chloro-1-naphthol in methanol and 5 volumes of TRIS buffered saline containing 0.003% H₂O₂.

Synthetic peptides

A synthetic 104mer peptide⁷ (104 amino acids from the C-terminus of p24) was assayed in both the Abbott and DuPont HIV ELISAs to detect their relative sensitivities to this gag protein. A synthetic RNase was used as a control preparation for these experiments.

To determine the key epitopes involved in binding, competitive blocking of epitopes was attempted using short synthetic peptides (15

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Figure 1 Results of testing dilutions of known standard in Abbott and DuPont ELISAs

amino acids in length). Each well of a polystyrene microtitre plate (Dynatech Laboratory) was coated with 50 μ l of 20 μ g/ml 104mer and allowed to dry overnight. The non-specific binding was then "blocked" with 5% BSA in PBS (two hours at room temperature) and washed with PBS plus 0.05% Tween 20 (PBST).

Each rabbit detector antibody solution (250 μ l) was preincubated at 37°C for two hours with 25 μ l of 50 μ g/l solution of short peptide, before 100 μ l of each mixture was added to each of the "104mer coated" wells and incubated at 37°C for one hour. After washing with PBST 100 μ l of a second detector solution (goat anti-rabbit horseradish peroxidase for the Abbott test; streptavidin-horseradish peroxidase for the DuPont test) was added to each well and incubated at 37°C for one hour. After a final wash with PBST 100 μ l of OPD was added and the reaction stopped after 30 minutes with 100 μ l of 1M H₂SO₄. The plate was read in the plate spectrophotometer at 492 nm. The whole experiment was done in duplicate.

Results

SENSITIVITY OF THE ASSAYS

Both tests showed similar sensitivities. Although the Abbott test appeared more sensitive, detecting 20 pg/ml p24, whereas the DuPont test could only detect 30 pg/ml p24 (fig 1), this difference was not significant.

The Abbott test quantified the level of antigen in U/ml of antigen, with a conversion factor of about 1.7 pg p24/U (table 1). The DuPont test claimed to give the result as pg p24, but calibrating against a known control it appeared to underestimate the amount of antigen (conversion factor 1.7 DuPont pg/pg p24), (table 1). Thus there was a threefold difference in the level of antigen recorded by the two assays.

SPECIFICITY OF THE ABBOTT AND DUPONT TESTS

Western blotting

From the manufacturer's data sheet, it seemed that the DuPont ELISA should just detect p24; the Abbott ELISA, using a polyclonal antibody against whole HIV, might detect other antigenic epitopes as well as p24. The results of the western blotting confirmed this (fig 2). The DuPont strip showed only one

Table 1 Calibration of the internal standards for the Abbott and DuPont ELISA tests

Abbott:			
	OD	p24 pg/ml	U/p24 ratio
500 U/ml	0.578	247	0.494
250 U/ml	0.352	127	0.508
125 U/ml	0.233	86	0.688
62 U/ml	0.125	36	0.580
31 U/ml	0.094	22	0.709
			(mean 0.6 U/pg p24)
DuPont:			
			pg/pg p24 ratio
125 pg/ml	0.673	184	1.472
62 pg/ml	0.379	102	1.645
31 pg/ml	0.206	55	1.645
16 pg/ml	0.119	31	2.060
			(mean 1.7 pg/pg p24)

band at p24. The Abbott strip showed a similar band at p24, but also bands at p18, p41-43, and a very weak band at gp120.

Synthetic 104mer peptide

The Abbott test could detect about 400 pg/ml of the 104mer; the DuPont test could detect about 100 pg/ml (table 2).

The Abbott test became saturated at 8 ng/ml 104mer, and despite raising the concentration to 1000 ng/ml the optical density did not approach the reading of the Abbott positive control.

In the DuPont assay about 1.3 ng/ml of the 104mer was equivalent to the 250 pg/ml positive control (or 480 pg/ml p24 using the conversion factor calculated above).

The results for the competitive blocking using short peptides are given in table 3. None of the short peptides affected the binding of the Abbott rabbit antibody to the 104mer. Three of the peptides partially blocked the binding of the DuPont rabbit antibody. These data suggest that there are binding sites for the DuPont antibody between the p24 amino acid residues 294-318 and 334-348.

Discussion

The results of this study suggest that the Abbott and DuPont tests have similar sensitivities. Although there are limited data on the relative sensitivities of the two ELISAs for the detection of antigenaemia, several studies have looked at the ability of the two tests to detect antigen in cell culture supernatant. Jackson *et al* evaluated the Abbott and DuPont antigen ELISAs and found that the Abbott test detected antigen earlier (mean 6.2 days compared with mean 7.4 days for the DuPont).⁸ The DuPont ELISA, however, was modified in December 1987 and it is not clear whether the original or modified ELISA was used in their study. Healey *et al*, using the original HIV antigen test showed that the DuPont could detect 70 pg/ml HIV protein (about 18 pg/ml p24); the Abbott test was slightly less sensitive and had a detection limit of 130 pg/ml (35 pg/ml p24).⁹

Barin *et al* and Crowe *et al*, (Programme and Abstracts of the IVth International Conference on AIDS; Stockholm 1988) studied the sensitivity of the two tests on serum samples and showed a discrepancy between the titres of antigen detected in the two tests. The Abbott

Figure 2 Results of western blotting both detector antibody solutions (A) Control (human HIV antibody positive serum); (B) Abbott polyclonal detector antibody; (C) DuPont monoclonal detector antibody.

Table 2 Comparison of ability of Abbott and DuPont ELISAs to detect synthetic 104 peptide fragment of p24

	Optical density			
	Abbott test		DuPont test	
	104mer	RNase	104mer	RNase
10 ⁶ ng/ml	0.677	0.045	> 2.000	NT
2.5 × 10 ⁵ ng/ml	0.638	0.044	> 2.000	0.037
8 × 10 ³ pg/ml	0.674	0.042	> 2.000	0.009
4 × 10 ³ pg/ml	0.410	NT	> 2.000	NT
2 × 10 ³ pg/ml	0.296	NT	1.642	NT
10 ³ pg/ml	0.170	NT	0.985	NT
500 pg/ml	0.116	NT	0.523	NT
250 pg/ml	NT	NT	0.280	NT
125 pg/ml	NT	NT	0.146	NT
63 pg/ml	NT	NT	0.081	NT
32 pg/ml	NT	NT	0.050	NT
Negative control		0.054		0.017
Positive control				
1000 U/ml (Abbott)		1.005		1.154
250 pg/ml (DuPont)				0.117
Cut off value		0.114		

Table 3 Results of blocking rabbit detector antibody solutions with short synthetic peptides before testing against 104mer peptide

Peptide	Abbott antibody Mean OD 0.452 (SD 0.028)	DuPont antibody Mean OD 0.952 (SD 0.022)
274–288	0.448	1.069
284–298	0.456	1.021
294–308	0.408	0.751*
304–318	0.401	0.779*
314–328	0.408	0.884
324–338	0.485	0.958
334–348	0.453	0.712*

test recorded twice as much antigen as the DuPont test according to Barin *et al.*, and three to nine times as much according to Crowe *et al.* In our study the Abbott “levels” were about three times those detected by the DuPont assay.

The result of the western blotting supports the view that the Abbott test detects other antigens apart from p24 of which the most important is probably the gp41–43 complex from the *env* gene. Goudsmit and Paul¹⁰ claim that the Abbott test will detect 50 pg/ml of recombinant core antigen, whereas recombinant envelope protein is only detectable at about 500 ng/ml, and that 90% of the rabbit detector anti-HIV activity is inhibited by core antigen, whereas only 8% is inhibited by envelope protein. Unfortunately, recombinant or purified envelope protein was not available for our study, and therefore these results could not be verified. Using the 104mer synthetic protein, however, the activity (judged from the optical density) was only 67% of the activity of the 1000 U/ml standard—that is, 0.677/1.005.

Similarly, the Abbott test was less sensitive than the DuPont assay at detecting the 104mer, and the binding of the DuPont rabbit detector antibody solution could be partially blocked using the synthetic short peptides. This sug-

gests that the Abbott and the DuPont tests recognise different antigenic epitopes, at least, on the core protein. This may in part explain the differences in the results of testing sera in the two assays. An alternative explanation for these results is that, although both Abbott and DuPont tests may recognise the same p24 epitope, the peptides failed to block the binding of the polyclonal Abbott antibody.

In conclusion, the two tests have different specificities and seem to detect different antigens. This may not be important in systems such as cell culture supernatant, where the antigens are produced in roughly fixed proportions. It may be very different in the detection of antigen in human sera, however, where the absence or presence of antibody may change the amount of antigen available for detection.

Similarly, the threefold variation in the quantification of antigen recorded by the two assays is disturbing. Again this is not as important if the tests are simply being used to tell if a cell culture is positive or negative for antigen, but may have repercussions if antigen titres are used to help decide on antiviral treatment. Conversely, in some clinical situations, such as determining whether primary infection has taken place, or in congenital infection, the antibody and antigen concentrations may be less important, and any concentrations of antigen be diagnostic.

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