

Blind comparison of Abbott and Dupont HIV antigen ELISA tests for detecting antigenaemia in asymptomatic human immunodeficiency virus antibody positive homosexual men

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Abstract

Three hundred and ninety eight serum samples from 270 human immunodeficiency virus (HIV) antibody positive asymptomatic homosexual men were tested in the Abbott and Dupont HIV antigen ELISA tests. In the Abbott test 62 (16%) of the sera were positive, according to the manufacturer's instructions, compared with 55 (14%) in the Dupont test. Twenty six sera were positive with the Abbott test but negative with the Dupont test and 19 sera were positive only by the Dupont test. Only 36 (9%) of the sera were positive in both tests. The Abbott confirmatory neutralisation test gave excellent agreement with the initial Abbott HIV antigen ELISA test; the Dupont confirmatory test was only in agreement with the initial positive Dupont antigen ELISA test in one third of the sera tested. Although the overall sensitivity of each of the two commercial assays tested was similar, the Abbott method may be preferable for clinical purposes if confirmation of an initial ELISA positive test result by neutralisation assay is required.

Human immunodeficiency virus (HIV) antigen tests are used to monitor patients with HIV infection who are receiving antiviral treatment.¹⁻³ They have been recommended as a useful index for initiating treatment.⁴ HIV antigen can also be detected in the serum before antibody seroconversion, but it then disappears in most cases as antibody titres rise.⁵ Patients who do not lose their HIV antigenaemia have been shown to be at greater risk of developing AIDS.⁶⁻⁸ Two commercially available HIV antigen enzyme linked immunosorbent assay (ELISA) tests (Abbott HTLVIII Antigen test and DuPont HIV p24 Antigen test) are widely used. In this study serum samples from asymptomatic patients with HIV infection were investigated for HIV antigen by both these tests. There was only "fair" agreement between the results of the two tests.

Methods

Between September 1987 and May 1988 all HIV antibody positive homosexual men

attending the sexually transmitted disease clinic at St Stephen's Hospital, Chelsea, were assessed, and those classified as the "CDC group 2" stage—that is, asymptomatic HIV infection⁹—were asked to contribute a specimen of blood for antigen tests. Three hundred and ninety eight specimens were obtained from 270 men. The samples were separated immediately and stored at -20°C until tested.

ABBOTT HIV ANTIGEN ASSAY

All the Abbott HIV antigen assays were carried out at St Stephen's Hospital where the results of the DuPont HIV Antigen assay were unknown. The sera were tested in the Abbott HTLV Antigen EIA (Abbott Laboratories, North Chicago, Illinois, USA) according to the manufacturer's instruction sheet. For each test a bead coated with human anti-HIV was added to 200 μl of serum and incubated overnight at room temperature. The beads were washed in distilled deionised water using Pro-Quantum (Abbott Laboratories) and reincubated with 200 μl of rabbit anti-HIV, for four hours at 40°C . After a final wash the beads were incubated with 200 μl of goat anti-rabbit horseradish peroxidase conjugate for two hours at 40°C . After the final wash the beads were transferred to tubes, 300 μl of substrate O-Phenyldiamine HCL (OPD) added, and the tubes incubated in the dark. The colour development was stopped after 30 minutes by the addition of 1 ml 1M H_2SO_4 , and the absorbance at 492 nm read using a Quantum Dual Wavelength spectrophotometer (Abbott Laboratories). A sample was regarded as positive if the optical density (OD) was greater than the cut off OD (0.05 plus the mean negative control OD).

The positive and equivocal sera (serum volumes permitting) were retested using the Abbott confirmatory test (Abbott HTLV III Antigen Neutralisation Test). In this test 200 μl of sample were preincubated with either 50 μl of Neutralisation Reagent (neutralised sample) or 50 μl of Control Reagent (control sample) for two hours at room temperature. After this a bead coated in human anti-HIV was added to each sample and the assay continued as for the HIV antigen test. Samples were considered to be confirmed positive if the control sample OD was greater than the mean negative control plus 0.04 and the reduction in the absorbance

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Accepted 21 December 1989

of the neutralised sample compared with that of the control sample was greater than 50%.

DUPONT HIV ANTIGEN ASSAY

All the DuPont HIV antigen assays were tested at the London Hospital Medical College where the results of the Abbott antigen assay were unknown. The sera were tested in the DuPont HIV p24 core antigen ELISA NEK-046 (EI Du Pont de Nemours and Co, Wilmington, Delaware, USA) according to the manufacturer's instruction sheet. The microtitre wells, coated with rabbit polyclonal anti-p24, were washed six times with the wash fluid provided, and incubated overnight with 200 μ l of each specimen, mixed with 20 μ l of 0.5% Triton X-100. The wells were rewashed six times and further incubated with 100 μ l of biotinylated rabbit anti-p24 for two hours at 37°C. After six further washes the antibody-antigen-antibody complex was probed by incubating with 100 μ l of streptavidin-horseradish peroxidase conjugate for 30 minutes at 37°C. After a final wash 100 μ l of substrate, (OPD), were added to each well, and the colour allowed to develop over 30 minutes, in the dark, at room temperature. The reaction was stopped by adding 100 μ l of IM H₂SO₄, and the absorbance at 492 nm was measured using a dual wavelength plate spectrophotometer (Dynatech MR 600) with a reference filter at 620 nm. The results were regarded as positive if the OD was greater than the reactive threshold value (mean negative control OD plus 0.1).

The positive and equivocal sera were retested using the DuPont confirmatory assay (DuPont) HIV p24 core antigen confirmatory assay A-112). In this assay 225 μ l of each sample were preincubated at 37°C for 30 minutes with 25 μ l of 5% Triton X-100 and of either 10 μ l of the confirmatory reagent (neutralised sample) or 10 μ l of the control reagent (control sample). After incubation 200 μ l of each mixture were added to each well of a prewashed HIV p24 antigen plate and the antigen assay continued as above. A result was positive in the confirmatory assay if the control sample OD was greater than the mean negative control plus 0.08 and the reduction in the absorbance of the neutralised sample compared with that of the control sample 7 was greater than 50%.

Various indices of agreement are available, but the κ index was chosen as it corrects for chance agreement between the assays. It takes the value of 1 when there is complete

Table 2 Comparison of results of repeat testing and confirmatory assays of both ELISA tests

Repeat testing	Abbott	DuPont
Positive on repeat testing/number of initial positive sera retested	53/54	52/53
Confirmation by neutralisation test:		
Positive on confirmatory testing/number of initial ELISA positive sera tested	53/53	15/46

agreement.¹⁰ Further details are available from the authors.

Results

The results of initial testing the sera in the individual HIV ELISAs are shown in table 1. In the Abbott test 62 of 398 (16%) sera were positive compared with 55 of 398 (14%) sera in the Dupont ELISA test. Only 36 of 398 (9%) sera gave positive results in both tests. Twenty six sera were positive with the Abbott test but negative with the Dupont test, and 19 sera were only positive by the Dupont test. The κ statistic only indicated "fair" agreement between the two tests ($\kappa = 0.549$, standard error (κ) = 0.050, $z = 10.9$; $p < 0.001$).

Both tests gave similar results on retesting the sera (table 2). The Dupont test gave poor correlation with the initial ELISA test as only 15 of 46 (33%) initial Dupont positive results were confirmed. In contrast, the Abbott confirmatory test confirmed 53 of 53 of the initial Abbott positive ELISA results. There were insufficient volumes of serum for Abbott neutralisation tests on nine samples.

Discrepant results were observed for a group of 18 sera which on initial testing were positive by the Dupont test but negative by the Abbott test. On confirmatory tests only one of the 18 sera was neutralised in the Dupont confirmatory test whereas the group that was both Dupont and Abbott positive on initial testing yielded 13 positive Dupont confirmatory results out of 30 tested.

Discussion

The overall sensitivities of the initial testing with Abbott and Dupont tests were similar, but there were great differences in the results obtained with individual patients. In the Abbott ELISA 62 of the sera from 44 men were positive, compared with 55 positive sera from 37 men in the Dupont test. Thus the prevalence of antigenaemia in the 270 patients investigated in this study was 16% with the Abbott ELISA and 14% with the Dupont test and is similar to that obtained in other studies. These range from 5% in asymptomatic Dutch patients (Abbott test)¹¹ to 19% in an American study (Abbott test).¹² The figures may vary with the type of subpopulation being studied, such as homosexual, haemophilic, or those with disease transmitted through blood products, and especially with the ethnic background of the patients being studied.^{13 14} In Boston six of 47 (13%) of asymptomatic HIV antibody positive homosexual men had antigenaemia by the Abbott test.¹² In Pittsburg HIV antigenaemia

Table 1 Comparison of results of initial testing of 398 HIV antibody positive sera by both ELISA tests

Abbott ELISA results	Dupont ELISA results		Total
	Positive	Negative	
Positive	36	26	62 Abbott positive
Negative	19	317	336 Abbott negative
	55 Total Dupont positive	343 Total Dupont negative	

occurred in eight of 52 (15.4%) subjects during the first 12 months after HIV antibody seroconversion.¹⁵

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 ELISA and found that the Abbott test detected antigen earlier (mean 6.2 days compared with mean 7.4 days for the DuPont).¹⁶ 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),¹⁷ whereas the Abbott test was slightly less sensitive and had a detection rate of 130 pg/ml (35 pg/ml). The results of the present study suggest that the Abbott test was more sensitive, in keeping with the findings of others who looked at the sensitivity of the two tests on serum samples (Abstracts presented to Fourth International Conference on AIDS, Stockholm, 1988).

There were many obvious differences between the results of initial Abbott and Dupont tests on individual patients. Only 36 of the 398 (9%) HIV antibody positive sera tested were HIV antigen positive in both tests, while 62 (16%) and 55 (14%) were positive in the Abbott and Dupont tests, respectively. This is clinically unsatisfactory—for instance, if the HIV antigen test were to be used as one of the variables for the start of drug treatment of HIV infection in asymptomatic patients different patients might receive treatment depending on whether the Abbott or Dupont tests were used. One possible explanation for the discrepant results between these two tests could be that each test detects somewhat different HIV epitopes. From the manufacturer's data sheet, the Dupont ELISA should just detect p24, whereas the Abbott ELISA, using a polyclonal antibody against whole HIV, may detect other antigenic proteins as well as p24.

Major discrepancies were also observed between the results of the Abbott and Dupont confirmatory assays. In the Abbott HIV ELISA 100% of the initial positive sera were confirmed positive in the Abbott neutralisation test whereas only 33% of the initial Dupont antigen positive sera were confirmed positive by the Dupont neutralisation test. Moreover, only one of 18 sera only initially positive by the Dupont antigen test was confirmed positive by the Dupont neutralisation test; nearly half the sera that were initially positive by both the Abbott and Dupont tests were confirmed positive by the Dupont neutralisation test. The clinical importance of Dupont positive HIV antigen results in sera where the Dupont confirmatory assay result is negative is unclear. The Dupont confirmatory test only required 10 µl of antibody solution incubated for 30 minutes with the serum sample. This confirmatory technique may not be adequate for neutralisation of the HIV antigen, or the Dupont HIV

initial assay may be capable of detecting the antibody-antigen complexes. The reason why some other sera gave confirmed positive HIV Abbott antigen test results but completely negative Dupont antigen test results could have been that the Abbott antibody system also binds to HIV proteins other than p24 while the Dupont detector antibody was specific for p24 alone.

The results of this study raise the clinically relevant question as to whether one or two different commercial tests should be used when an HIV antigen test is requested as the use of only one test method will "miss" a significant number of positive results detected by the other method. Ideally, both Abbott and Dupont antigen test results would be given for each serum, but this policy would be both technically labour intensive as well as expensive. Even if both tests were to be used there would be occasional problems in interpreting the results as we are not sure why the tests sometimes give different results. In our opinion the serum reacting as positive by both tests could be regarded as a true positive result, while a sample reacting positive by the Abbott test alone could be regarded as a positive result, although with rather less conviction. A sample positive by the Dupont test alone—that is, no confirmation forthcoming, cannot be regarded as positive from the point of view of clinical evaluation until further information is available about the clinical importance of an Abbott negative/DuPont positive test. If only one commercial assay is used it is clearly desirable to use a test where a positive result can also be confirmed by a neutralisation test—thus the Abbott method may be preferable to the Dupont test in this regard.

We are grateful to Dr A J Nunn for help and advice with statistical analysis and to Farmitalia Carlo Erba for financial support.

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