AIDS: The Safety of Blood and Blood Products
Edited by J. C. Petricciani, I. D. Gust, P. A. Hoppe and H. W. Krijnen
© 1987 by the World Health Organization
Published by John Wiley & Sons Ltd

16

Current Anti-LAV/HTLV-III Screening Methods (and some of their problems)

JOHN A. J. BARBARA
Head of Microbiology, North London Blood Transfusion Centre, Deansbrook Road, Edgware, Middlesex, HA8 9BD, Great Britain

AIM OF ASSAY

Screening tests for anti-LAV/HTLV-III became regularly available for routine use in the middle of 1985. Presence of antibody to LAV/HTLV-III in a donor provides an indication of infection with the agent and an ensuing risk of infectivity. A striking aspect of the introduction of the tests was the speed with which they were developed and the comparative effectiveness of most of the commercially produced kits. Because they were urgently required, they needed rapid evaluation. The only assays so far available for mass routine use have been of commercial origin. These are based on infected cell lines licensed to different companies in various countries by the research teams who developed them. Naturally the tests are not perfect, and some of the problems associated with their use will be discussed. However, a detailed catalogue of the characteristics of each of the many tests available is not provided since they are continually being modified by the manufacturers and several new tests are being developed.

Theoretically, a direct approach to screening for infectivity might be to detect viral antigen(s), and in the future, viral nucleic acid detection may be

WITN6989009_0001

ANTI-LAV/HTLV-III SCREENING METHODS

The requirements for a good screening test in the context of blood collection centres are: sensitivity, specificity, speed, cost-effectiveness, simplicity and convenience. It is encouraging that commercially available assays have gone a long way towards meeting these exacting and conflicting requirements so quickly. In particular, increasing sensitivity is often associated with a corresponding decrease in specificity and tests have to be 'gated' to achieve an optimum balance. In this respect our experience suggests that the competitive type of assay is especially successful.

The antiglobulin format is shown in Figure 1. Because LAV/HTLV-III replicates by budding through the cell wall, it carries cell antigens with it; in addition to this, viral purification for preparation of reagent antigen can never be perfect. Therefore, nonspecific but repeatable 'cross-reactions' can occur, as shown in Figure 2. Sayer *et al.*[6] concluded from a study of 15,680 volunteer blood donors that 'HLA antibodies are an important cause of false-positive reactions in the screening test'; they attributed three-quarters of the false-positive reactions to HLA antibodies. In Sayers' study most of the HLA

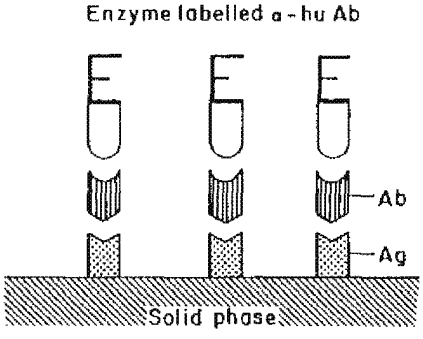

Fig. 1 'Antiglobulin'-type assay

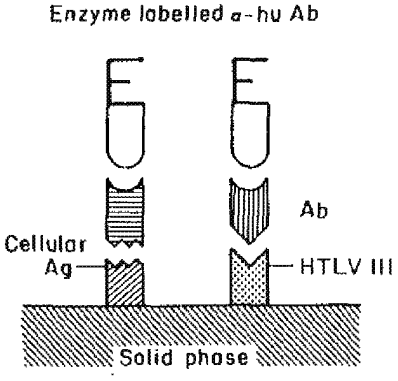

Fig. 2 Cross-reactions

It would be inappropriate to attempt a detailed description of all the variables involved with commercially available kits. Competitive assays benefit from absence of a sample pre-dilution step and the ensuing speed and simplicity resulting from this. The dilution factors involved, the incubation times, and the type of substrate employed vary with different antiglobulin kits. A non-carcinogenic substrate such as tetramethylbenzidine is preferable if the increased safety claimed for ELISAs is to be realized. Assays with appreciable false-positive rates due to 'sticky sera' or susceptibility of HLA cross-reactions are very dependent on the availability of definitive confirmatory tests; these confirmation tests are not yet completely satisfactory. With competitive assays, special attention must be paid to the plate washing to avoid false-negative results. In this respect, computer-generated histogram analysis of each plate's results (Figure 5) has proven extremely useful, and in our experience should be generally recommended. Nevertheless, in areas that cannot afford plate readers the competitive assay proved convenient for reading 'by eye' in a trial comparing machine and visual analysis of results (Dr R. Tedder, personal communication). In this situation it is preferable to stop the reaction with sodium fluoride as opposed to acid, so that the blue colouration of the reaction mixture is not turned to yellow.

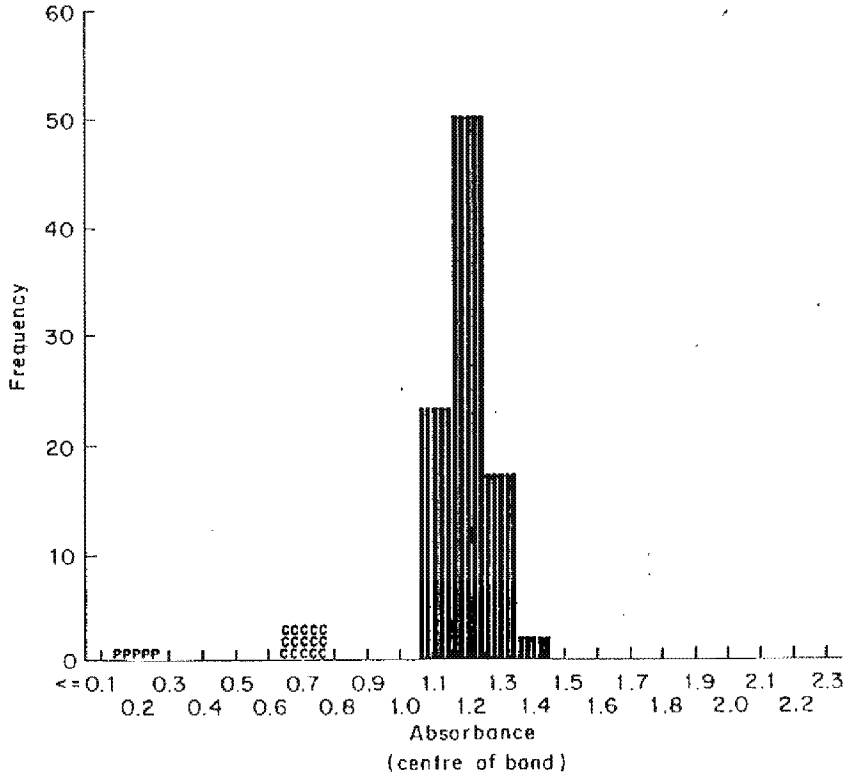

Fig. 5 Computer-generated Histogram analysis, C = kit cut-off control sample; P = kit positive control sample; — = negative control sample

WITN6989009_0003

Table 1 Anti-LAV/HTLV-III screening in the United Kingdom (provisional figures; 1 million screen tests)

Test	Screen pos./equiv.	Repeat pos./equiv.	Confirmed pos.
Wellcome (778,000)	1 in 210	1 in 11,300	1 in 56,000
Organon (274,000)	1 in 160	1 in 680	1 in 55,000
1,052,000			1 in 55,000

at the Manchester Transfusion Centre the results of antibody screening in the United Kingom. A similar low prevalence is shown with either the Wellcome competitive assay or the Organon anti-globulin test. Currently, the antibody prevalence at the North London Blood Transfusion Centre, where a competitive assay is used, is approximately double that for the UK overall. The difference in repeatedly positive or equivocal results by the two tests is very marked, being much less with the competitive type of assay. It may be better to avoid excessive numbers of false-positive samples rather than attempting to eliminate them by sometimes equivocal confirmation tests.

ASSESSING SENSITIVITY OF SCREENING TESTS

Accurate and fair assessment of different kits is difficult, but may be approached by examining panels of 'pedigreed' anti-LAV/HTLV-III-positive samples, performing end-point dilution studies or testing serial samples from patients during the period of seroconversion. Generally the tests are mostly of satisfactory sensitivity, although there are no extensive data on the distribution of naturally-occurring antibody titres from different anti-LAV/HTLV-III-positive subjects. This would be valuable information to have in case there is a preponderence of infected subjects with low levels of antibody, or vice-versa.

In general terms the tests are sensitive enough to detect anti-LAV/HTLV-III in immunoglobulin preparations prepared from pooled plasma, although the approximately tenfold concentration of the IgG in such preparations may interfere with testing. In a retrospective study, a competitive assay was also able to detect passively transferred antibody in a patient some days after transfusion of a platelet preparation, subsequently found likely to have been anti-LAV/HTLV-III positive (unpublished results). However, it appears that different tests may show variations in their ability to detect antibodies to different components of the virus; current competitive assays seem better than certain antiglobul in kits in detecting antibody at the point of seroconversion. Conversely, antiglobulin kits may have the edge in end-point dilution studies. Recent speculation about new LAV/HTLV-III strains[11] emphasizes

WITN6989009_0004

J. D. (ed.), *Progress in Transfusion Medicine*, vol. 1. Churchill Livingstone, Edinburgh.
6. Sayers, M. H., Beatty, P. G., and Hansen, J. A. (1986). HLA antibodies as a cause of false-positive reactions in screening enzyme immunoassays for antibodies to human T-lymphotropic virus type III. *Transfusion*, **26**, 113–115.
7. Karpas, A., Gillson, W. Bevan, P. C., and Oates, J. K. (1985). Lytic infections by British AIDS virus and development of rapid cell test for antiviral antibodies. *Lancet*, **2**, 695–697.
8. Cheingsong-Popov, R., Weiss, R. A., Dalgleish, A. *et al.* (1984). Prevalence of antibody to human T-lymphocyte virus type III in AIDS and AIDS-risk patients in Britian. *Lancet*, **2**, 477–480.
9. Holland, P. V., Richards, C. A., Teghtmeyer, J. R. *et al.* (1985). Anti-HTLV-III testing of blood donors: reproducibility and confirmability of commercial test kits. *Transfusion*, **25**, 477–480.
10. Schorr, J. B., Berkowitz, A., Cumming, P. D., Katz, A., and Sandler, S. G. (1985). Prevalence of HTLV-III antibody in American blood donors. *N. Engl. J. Med.*, **313**, 384–385.
11. *Nature* (1986). News: AIDS research. *Nature*, **320**, 385.

DISCUSSION

Screening Tests

Dr Habibi

I would like to comment on the definition of specificity and sensitivity as addressed by Dr Allen. If I understood correctly, Dr Allen and many other researchers in the US, and sometimes in Europe, define sensitivity and specificity in terms of infection. However, Dr Allen himself rightly stressed that we cannot define the infection with 100% reliability. Now, the screening ELISA test, as well as the reference Western blot or RIPA test as we use them presently, aims at the detection of a precise viral antibody, i.e. anti-LAV/HTLV-III. So, with this view in mind I think that the sensitivity and specificity of these tests should be assessed solely on a serological basis, leaving aside the natural history of infection by this virus. Two or three clinical settings seem to me worth emphasizing with this respect. First, those acutely infected patients with the AIDS virus during the incubation period who are seronegative but virus culture-positive. Two, those full-blown AIDS patients at advanced stages of the disease who sometimes become seronegative while definitely infected with the virus. In this latter setting particularly, we can hardly put the blame on the test and label it insensitive for the detection of infection. So, may I suggest that for clarity of discussions 'false-positive' be defined as a positive reaction *without*, and 'false-negative' be defined as a negative reaction *with*, the presence of LAV/HTLV-III antibody detected by reference methods presently available.

your initial test. Now the difference between the competitive assay and the antiglobulin assay is that when you take out those samples and check them again, with the competitive assay you can eliminate those borderline samples which have come up because of operator or machine factors; you are then not left with a large number of repeatedly reactive positive samples to be sent on for confirmation. That has been a very clear difference in all the laboratories that have been using the two tests in Britain. The other question concerns the quality control samples that are included. When the tests are all 'new', and our understanding is less than complete, one errs on the side of safety and intersperses the control samples liberally to make sure that the test is working nicely across the plate. So initially, larger numbers of quality control samples will be used.

Dr Pindyck

A question was raised concerning the reproducibility of licensed assay systems and experience in field conditions. Let me just briefly report to you on our experience with almost 1 million screening tests on blood donors in our region. We use an antiglobulin microtitre plate system. Seventy percent of our initial reactives are repeatable. Those which are not, are in the very low OD range. Our system has a fixed cut-off of 0.1. Those which do not repeatably react have an OD of less than 0.2. Our correlation of initial to repeat testing is better than 0.9, and of the two repeat tests it is better than 0.98. We consider that the tests, at least in our hands, are certainly suitable for field operations. This particular system is particularly suitable for confirmation of sample identity as well.

Dr Davey

Could I ask Dr Zuck if he foresees that the authorities will eventually have faith in the false-positive, as it were, and let us readmit these to the ranks of donors?

Dr Zuck

I think it is inevitable that this issue should come up in this forum, and I would be very interested in the opinion of people who have an enormous amount of experience with the test and who are gathered in this room from around the world today. There has been a scheme proposed in the USA which I will describe for people to consider and discuss. It has been proposed with the increasing quality of the tests – and some of the newer tests appear to be somewhat more specific – that if a sample is repeatably reactive and is Western blot, RIPA and/or IFA negative, whichever of the confirmation

WITN6989009_0006

is that perhaps the scheme I proposed, which has been discussed at great length in the USA, could, with some additional modifications, increase the security that we would not be reinstating an infected donor.

Dr Deinhardt

In principle, I agree with Dr Zuck. This scheme is approximately comparable to the scheme which we have in the Federal Republic of Germany. However, the scheme will probably be revised once the recombinant ELISA test satisfies without question the requirements for adequate sensitivity and specificity. To the second point Dr McClelland made: In the case of a repeatedly ELISA-positive but Western blot-negative sample, we can usually identify non-specificity by immunofluorescence staining of non-infected cells, and we can identify non-specific bands in immunofluorescence using a mock antigen. Under such conditions we can be satisfied that the blood is 'true false-positive' or that another is 'true-negative'.

Dr Barbara

I think part of the problem is the question of the confidence that you have in the kit that you are using. I think the tests are evolving and I am surprised that I have not heard any data concerning, for example, the use of uninfected cell extract to absorb out cross-reactions, followed by retesting. This might be a better alternative to that of just using uninfected control cell plates if a reaction is found (assuming there is no specific anti-LAV/HTLV-III at low levels). The other point that strikes me about this is that some time you hopefully are going to have to rely on tests that have a lower rate of positives. Obviously my experience is biased by a test that has a low rate of repeatable positives. But what happens in a centre when you have been using a test with a lot of repeatable positives which are Western blot-negative, and then you switch to a test with far fewer repeatable positives? Does this imply that you have less safety now, and that all the time beforehand you were being safer because you were excluding those extra donors? Or does it really just imply that you were excluding a lot of people unnecessarily?

Dr Deinhardt

In respect to what Dr Barbara has just said: It's not just taking an ELISA plate coated with non-infected cells. I am talking about mock immunoblots where one can identify exactly the proteins with which these sera react. Another point: we have found in comparing a very large number of test kits from different manufacturers – with the exception of those which use LAV grown in different cells and which have more recently come on to the market