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WHAT'S NEW IN TESTING FOR ANTI HIV?

Since the first commercial anti HIV assay was introduced in UK in April 1985 the pace of innovation has been remarkable. New products, many of them incorporating interesting developments or modifications of existing technology, have appeared every month and this process has been fuelled by the desire of companies to establish a foothold in a very competitive prestige market. In UK the absence of formal licensing procedures has encouraged the companies to bring new products quickly to the market and a wide array of commercial HIV tests has been presented to British customers. Through Department of Health funded and other published evaluations laboratories have been kept informed about most of these assays, and over a dozen of them, led by Wellcozyme, Abbott EIA and the Serodia particle test, are in current use here. This paper summarises these developments up to the latter part of 1989.

New viruses: No account of HIV tests can now ignore the discovery and characterisation in 1985/6 of a second AIDS virus, HIV 2, or the fact that other HIV infections are being found which give rise to intermediate serological findings. These might be dual infections with HIV 1 and HIV 2, but perhaps more frequently they are infections with a single variant HIV strain. More minor and possibly major variants of HIV are likely to be recognised in the future.

The discovery of HIV 2 is having a significant effect on testing procedures and policies. This virus is particularly prevalent in parts of West Africa, but it has been found in former Portuguese colonies in other part of Africa (Angola, Mozambique), in Portugal itself and in other parts of Western Europe. About seven HIV 2 infections have been reported in England, and one blood donor in London was recently found to be anti HIV 2 positive. There will therefore be an increasing need for 'combined' anti HIV 1/HIV 2 screening assays and for serological tests that distinguish HIV 1 from HIV 2 infection (see below).

New assay formats: The first anti HIV ELISAs were all of the indirect format in which a reaction between the specimen and the antigen on the polystyrene solid phase is detected by the addition of enzyme conjugated antibody to human immunoglobulin. These assays have some shortcomings e.g. a need to dilute specimen, multiple steps and non-specificity. Alternative ELISA systems ('competitive', 'sandwich', 'capture') have since been developed and these offer certain advantages e.g. simplicity, specificity and applicability to a wider range of specimens. Good competitive assays are available from Behring, Organon and Wellcome, and Wellcome (who have been very innovative in this area) are currently developing sandwich and capture ELISAs.

With simplicity and speed in mind other companies have developed products with alternative solid phases to polystyrene. For instance Fuji has an excellent test based on minute gelatin particles coated with HIV antigen that agglutinate in the presence of anti HIV (Serodia). Cambridge Bioscience has developed a latex slide agglutination assay which produces a result in a few minutes. Other commercial tests based on more novel solid phases have also appeared. The first ones involved the application of native viral protein to nitrocellulose strips (Western blot). More recent ones explore the application of 'recombinant' proteins or synthetic peptides to nitrocellulose (immunoblot) and the use of a porous HIV antigen-coated membrane (e.g. HIV-Chek) through which reactants and washing buffers can be

poured. This last system is presented as a small, single-sample cartridge and provides a rapid result based on a colour change on the membrane (HIVchek, Test-Pak). These new non ELISA tests form a group of simple to use and mostly rapid assays conveniently applied to small numbers of specimens and not requiring semi-automated machinery such as washers and spectrophotometers. They are all read by eye. They are in general less accurate than ELISA and some still require further development; but they have potential applications both in confirming reactivity and where ELISAs would be inappropriate or inconvenient.

New Reagents: The most far reaching development in HIV testing since 1985 has been the introduction of genetically engineered antigens for anti HIV assays. They are of two kinds: firstly there are polypeptides of a few hundred amino acids. These are the products of expression vector systems into which HIV gene sequences have been inserted and expressed. The recombinant polypeptides are coded by relatively large parts of HIV genes and are likely to have the structure and configuration as well as amino acid sequence of the native protein. They are therefore likely to behave very similarly to it. Secondly, there are oligopeptides, typically of 10 to 30 amino acids, which probably only represent one or two epitopes of the native protein antigen on which they are based and which must be very carefully selected if they are to mimic the corresponding native protein.

These two kinds of HIV antigen, recombinant polypeptide and synthetic oligopeptide, offer great advantages over live virus-derived antigen. They are safe to prepare; they can be produced in limitless amounts quite cheaply; and they are precisely characterised and therefore can form the basis for specific assays for antibody to particular proteins of HIV 1 or HIV 2 as well as for screening assays. They are free of human cellular antigen, though non-specific reactions between specimen antibody and, for instance, vector yeast protein may still occur. Generally, the commercial assays in which they have been incorporated are just as sensitive and rather more specific than the same assays based on native antigen would be.

The breadth of antigenic 'cover' that these engineered antigens provide remains in some small doubt, however. It is conceivable that there are individuals whose antibody response to HIV infection is so narrow that their serum would not react with the limited range of mainly envelope and core antigens represented by the recombinant polypeptides and synthetic oligopeptides in the new assays. To exclude this possibility as extensive an evaluation as possible of each one of these genetically engineered products ought to be done while large numbers of positive sera diagnosed by assays based on native viral antigen are still available. This may entail testing a thousand specimens from seropositive individuals together with as many sera as possible from those in the course of seroconversion. Where this has already been done most of the recombinant polypeptide-based products, e.g. Abbott, Wellcome, and some of the oligopeptide-based products, e.g. Behring, have performed as well as or better than assays based on native virus. With careful choice of recombinant and synthetic peptides that have been evaluated by extensive empirical testing it would therefore seem the use of antigens prepared from live virus could be discontinued with several consequent benefits.

Rapidity of new tests: The difference in time taken to complete various anti HIV tests has already been touched on. The need for speed arises in several situations: in transfusion it allows rapid donation processing and release; in diagnosis it provides quick reassurance to the patient (the concept of the same day anti HIV result is gaining ground); in transplantation and other surgical procedures testing may determine whether urgent work goes ahead. Two distinct requirements emerge from this: firstly, for a rapid, very sensitive ELISA for use on large numbers of specimens in transfusion laboratories and, secondly, for a fast and simple test applicable to a few specimens at a time. It has not so far been possible to make a really sensitive ELISA that can be completed in less than two hours. Two problems are that 'early' non-avid antibody does not bind rapidly to antigen and that the chromogenic enzyme systems used in conventional ELISAs cannot be further speeded up. The introduction of a novel, almost instantaneous detector system, the Amersham chemiluminescence test, is of interest because it eliminates the slow colour generation step of other ELISAs; however it requires an expensive reader to measure the end point of the reaction.

Truly rapid assays e.g. latex agglutination, HIV-Chek, can generate results in less than 30 minutes; but against this must be set their relative insensitivity and the strict limit to the number of specimens that can be handled at once. There is also uncontrollable variability with the skill of the operator who performs and reads the test. These assays may be helpful for the single emergency specimen, but conservative opinion at present favours the use of the best current ELISAs or the simple and very sensitive Serodia particle agglutination assay whenever the necessary two hour wait for results is acceptable.

New Confirmatory Tests: As already pointed out, HIV 2 is more prevalent than HIV 1 in some West African countries. It is nearly as prevalent as HIV 1 in several more, and is an occasional infection elsewhere in Africa and Europe. HIV 2 is likely to establish itself as a major or minor cause of AIDS in many countries. This situation has changed confirmatory testing firstly by adding a new possible cause for reactivity in HIV screening assays and secondly by raising the difficult question of which is the infecting virus.

The conventional answer both to confirming any reaction in an HIV screening test and to discriminating between HIV 1 and HIV 2 infection is to resort to Western blot. However, several problems arise. Firstly, a high proportion of normal specimens give isolated reactions e.g. to p24 and so it has proved difficult to define accepted criteria for Western blot positivity. Secondly, the distinction between HIV 1 and HIV 2 infection on Western blot, while easy for some specimens, is difficult when antibody responses are so strong that cross reactivity obscures the basic differences in the positions and intensities of the anti gag and anti env reactions. Thirdly, the cost of this procedure, especially if follow up and repeated testing is needed, is excessive. More than £100 is frequently spent on reagents alone to determine whether an ELISA screening test reaction is specific and whether it is due to HIV 1 or HIV 2.

Fortunately, the recent development of genetically engineered peptides that have the same epitopes as individual native HIV 1 and HIV 2 proteins can resolve this problem if it is allowed to do so. Immunoblot strips are now available to which the individual synthetic peptides have been applied as bands, both in the same form as regular HIV Western blot strips and in a form that will distinguish HIV 1 from HIV 2 (e.g. Peptilav). Alternatively, individual recombinant peptides of HIV 1 and HIV 2 can be bound to the

internal surfaces of columns of wells in a microtitre plate to provide a confirmatory ELISA (Beckmann). There are several advantages in this approach. The genetically engineered products are easier, cheaper and safer to produce than Western blot, and more reproducible. Reactions with them may be measured colorimetically at the positions where the synthetic antigens have been applied, and so objective criteria can be set for confirming HIV positivity and distinguishing between anti HIV 1 and anti HIV 2 reactions. Finally, the susceptibility of Western blot to non specific anti-cellular reactions e.g. false anti p24 or anti p55 is less likely to be a feature of confirmatory tests based on these 'designer' proteins. The FDA has so far been very reluctant to license anti HIV screening kits that are not based on native proteins. However, the best of the new generation of anti HIV screening assays with engineered peptides are proving so reliable that this stance will have to change, to be followed in my opinion by a similar acceptance of genetically engineered products as the basis for confirmatory assays.

New evaluations: In conjunction with the Procurement Directorate, Department of Health, the PHLS Virus Reference Laboratory has evaluated most of the new anti HIV assays reaching the British market. The most recent evaluation, now almost complete, has been of combined anti HIV 1/HIV 2 assays. Combined assays are now to be preferred for screening clinical specimens in UK and, as long as they prove sufficiently specific, are likely to be introduced to screen UK blood donations. Several combined products e.g. the new Behring, Abbott and Wellcome assays seem promising but the results of new batch of evaluations are not yet fully analysed. They will be available at the beginning of 1990.

It should be understood that these evaluations do not constitute a licensing procedure. The intention is to identify unsatisfactory assays to laboratory managers and to provide initial data on a range of satisfactory new products which merit consideration. The results should be taken with other published evaluations and local experience as the basis for making a particular choice. DoH/PHLS evaluations do not attempt to identify a single 'best buy'.

New performance assessment: In UK the main plank of quality control in HIV testing has been the distribution of coded panel of specimens from the PHLS Division of Microbiological Reagents and Quality Control (DMRQC) to laboratories participating in the NEQUAS scheme. Other coded and uncoded panels have been issued regularly to the Blood Transfusion Service and recently DMRQC has begun, through WHO, to make similar panels available to laboratories abroad. There are three NEQUAS distributions each year and other specimens are always available for control purposes. This level of activity in external quality control is an important feature of HIV testing which needs to be strengthened. The distributions are costly, but they encourage critical self-assessment by laboratories and provide the justification for external bodies to intervene and try and raise standards where these are low. In the field of HIV, internal and external quality control will be at least as important as further technical development in improving the accuracy of testing.

New Specimens and new applications: In epidemiologic investigations techniques are needed that allow accurate results on single specimens. This requirement arises particularly in anonymised investigations where no follow up is possible and where the specimen may for instance be a small blood sample collected onto filter paper from a heel, finger or ear prick.

Developing suitable anti HIV technology for such specimens has been made difficult by delays in winning ethical approval for anonymised studies. This has meant that almost no pilot studies have been possible. However, one large study of heel prick specimens in London has been successfully done using a modification of the Serodia test, the outstanding sensitivity of which has already been referred to. Serum based anonymised studies will not require anti HIV assays with such high sensitivity, but excellent specificity and the ability to detect anti HIV 2 as well as anti HIV 1 will be needed. It is as yet unclear which assays are most appropriate for anonymous studies in UK, though there are several strong candidates.

In collaboration with Wellcome Diagnostics, the PHLS Virus Reference Laboratory has been developing an ELISA that will detect, with the accuracy that secure diagnosis demands, anti HIV in saliva, urine and blood spots. A prototype kit is currently being assessed in six UK laboratories. Findings in the Virus Reference Laboratory are very encouraging on the critical points of specificity and sensitivity for low concentrations of antibody in specimens like urine. However, full evaluation of the test and the development of satisfactory collection methods, e.g. for saliva and finger pricks, are still awaited.

Summary

There are continuing improvements in anti HIV testing. These reflect big investments in research and development by large and small companies in the field of diagnostics. The benefits to the patient, the clinician and the researcher are being seen in more accurate, rapid, objective and versatile anti HIV assays. It is now likely that errors arising in laboratory diagnosis are attributable to human, not to product failures. Therefore, while we may continue to press for further improvements in the quality of anti HIV kits, there should be no illusions about where the source of errors is likely to lie. Anti HIV tests are being done in 140 laboratories that participate in the UK NEQUAS scheme and in some others that do not. More emphasis on training and on internal and external performance assessment is needed if the fullest benefits of good research and development in this field are to be realised.

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Recommended reading

1. Morbidity and Mortality Weekly Report 38 s7, pages 1-6 (21 July, 1989) (describes criteria for Western Blot positivity).

2. Genesca J et al. What do Western blot indeterminate patterns for HIV mean in EIA negative blood donors? Lancet (1989) ii; 1023-25.

3. HIV Detection by Genetic Engineering Methods ed. PA Lucin and KS Steiner published by Dekker, New York, 1989.

4. Report of DoH Procurement Directorate/PHLS Virus Reference Laboratory Evaluation of combined anti HIV1/HIV2 assays: in preparation.