Ten years of laboratory diagnosis of HIV: how accurate is it now?

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The high expectations of laboratory diagnosis of HIV have mostly been met, but some problems remain. Current tests for anti-HIV antibodies are sensitive enough for many purposes, but for earliest diagnosis direct tests for virus are necessary. False negative results may occur, for instance because infection with virus variants such as HIV 1 subtype O is not recognised, or because of laboratory or clerical error. The laboratory monitoring of mothers and babies who have been treated with zidovudine, to establish whether perinatal infection has taken place will be difficult, and more reliable quantitative assays of HIV are needed to measure the risk of transmission from mother to baby. Proposals to introduce home testing for HIV may improve ascertainment of infection, but there must first be adequate support in place for those individuals who find themselves HIV-positive. Quality assurance, especially through clinical and laboratory audit, may now add more to diagnostic accuracy than the development of even more sensitive assays. The outstanding challenge is to make universally available tests with the accuracy and consistency achieved when best laboratory practice is enforced.

Introduction

It would be easy to be complacent about the laboratory diagnosis of HIV in the United Kingdom. After a decade of intensive work and abundant public and commercial investment a diagnosis can be made in most circumstances, with an accuracy that is unsurpassed in other infections. Mainly as a result of this, the number of known transmissions from UK blood donors to recipients since anti-HIV screening began in 1985 is negligible and there are almost no known instances of UK blood donors having been erroneously told that they are infected with HIV. Indeterminate and equivocal results given to blood donors have also been minimised. Clinical diagnosis has probably not been quite as accurate, though very few false positive results have been detected in follow-up of patients.

To be set against these achievements, however, are some problems in HIV diagnosis that remain unresolved. In this paper I seek very briefly to review five of these problems: (i) early infection events, their recognition and the implications for blood, tissue and organ donation; (ii) HIV variants and their potential for giving rise to false negative results; (iii) the problem of laboratory error; (iv) the issues of antenatal and infant testing; and (v) the prospect of home testing for HIV infection. These are matters that pose difficult technical, logistical and ethical questions and, as they are unlikely to be
resolved quickly, it is important to be aware of them. Once they are resolved, there will be opportunities for more effective HIV prevention and management.

**Tests for HIV**

Antibody tests remain the cornerstone of HIV diagnosis. They are widely available, rapid and highly accurate. It has been estimated recently that an HIV carrier makes between $10^7$ and $10^8$ new virions each day, and with this immune stimulus in mind one would expect to be able to detect a sustained anti-HIV response. Other diagnostic methods, e.g. p24 antigen testing, and proviral DNA and RNA amplification exist, but these innovations in HIV diagnosis need to be matched against the anti-HIV test and should be rejected unless they fulfil a need that antibody testing fails to meet. Most of the five problems I have enumerated above are ones where ordinary antibody testing alone have not been adequate, and where the solutions lie in finding suitable additional tests. In each case, however, these supplementary techniques are likely to increase the cost of consumables by five to 50-fold. The more effective use of antibody testing (if possible) is likely therefore to be the most economical solution.

**Early events in HIV infection**

Serological tests cannot be effective very early in infection before antibody appears. For most people, the moment at which they contract HIV is unrecognised at the time often happening in intimate or even illicit circumstances. Thus the early development of HIV infection is still, after 10 years, poorly studied. Careful observation of seroconversions in men at risk (Busch et al., 1995), and in blood donors and the relatively few cases of inadvertent transmission to blood recipients (Petersen et al., 1994) has established the intervals between the moment of infection and the appearance of new markers for HIV compared with the viral lysate anti-HIV assays in use pre-1990. In individual cases the best anti-HIV assay can bring forward detection by 20 days. The most sensitive assay, PCR for HIV RNA, may improve this by a further 10 days (Table). Of course, these data do not tell us how long the latent period may be between exposure to HIV and the appearance of the earliest marker of infection. Nor do they reveal which factors determine whether an exposure leads to infection. It may be assumed that the route and the nature of exposure (blood or other body fluids, sexual, mother-to-child or parenteral) and the dose are important variables, and it has also been hypothesised that some quasi-species of HIV 1 are more transmissible than others. But data, as opposed to supposition, are lacking and the means of measuring dose and characterising HIV 1 quasi-species are not well developed. It is known that in other virus infections, e.g. hepatitis B and rabies, the route and dose are important variables determining transmission, but generally the factors that cause some sexual, mother-to-child and blood exposures to HIV to result in infection, and others that do not, are poorly defined. In particular, an affordable and reproducible test that would easily quantify infectious HIV to the nearest log_{10} concentration is needed to study the transmissibility of the virus from carrier to susceptible contact.

Studies of seroconverting blood donors and recipients have shown that HIV p24 antigen detection and gene amplification methods such as PCR have not greatly shortened the interval between exposure and first detection of infection. They would therefore seem to add little to the screening of empanelled blood donors or, in
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the UK, of new blood donors. UK donors are selected from a population with a low prevalence of HIV infection and this augments the safety of blood for transfusion. Tests other than for antibody will add little to this. A different issue is whether PCR or antigen tests can improve the safety of tissue and organ donations in the UK. For these, the selection procedures applied to blood donors have to be modified. In particular, cadaveric donors can not be preselected in the way that blood donors are, and follow up tests cannot be done. Transplantation is becoming more common and, compared with blood donation, is a poorly regulated field. Each type of procedure needs to be assessed to determine which tests will minimise the risk of HIV transmission. The donor history, if available, access to an ante- or post-mortem blood specimen, the urgency of the procedure and the scarcity of transplantable material are other factors to be considered.

HIV variants and false negative results

It is well known that HIV has two subtypes, HIV 1 and HIV 2. The latter was first recognised in France in 1986, and has since been diagnosed in West Africa, Mozambique, Angola, Portugal and, to a lesser extent, elsewhere in Europe. In the UK it has been diagnosed in about 1 in 600 HIV infections (J. Y. Mortimer, PHLS Communicable Disease Surveillance Centre, personal communication). As the morbidity, mortality and transmissibility of HIV 2 are all less than that of HIV it is in every sense a more minor problem (de Cock & Kanki, 1994). Its recognition by 'combined' anti-HIV assays appears to be effective (though this has never been formally investigated), and it can be distinguished from HIV 1 either by Western blotting or, much less expensively, by the use of the more specific 'competitive' screening assays for anti-HIV 1, or specific anti-HIV 1 and anti-HIV 2 gel particle-based assays (Parry et al., 1995).

In recent years, HIV 1 subtype O (a group of 'outliers' of HIV 1) has been described by Belgian, German and French investigators. To date, these virus infections have been found only in Cameroon, and in small numbers, but they may not be detected by current anti-HIV 1 or 2 assays. Claims have since been made by leading manufacturers that the antigenic make-up of their assays has been suitably adjusted to detect anti-HIV 1 subtype O, but it is impossible to test these claims effectively and there is a small risk that these adjustments might have compromised the accuracy of the assays tests for the predominant virus, HIV 1. Once detected, confirmation of HIV 1 subtype O infection by Western blot is straightforward, though reportedly the p160 band may not be as strong as the p24 and p31 bands (N. Paternoster, personal communication).

<table>
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<th>Table Estimated reduction in the 'window' period from the time of appearance of anti-HIV detected by a viral lysate antigen based assay (Petersen et al., 1994; Busch et al., 1995)</th>
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<td>Best of current anti-HIV assays</td>
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<td>p24 Ag</td>
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The key question for national authorities with regard to HIV 1, its outliers and HIV 2 is whether the screening tests are detecting all the infections that arise in each country. This question is unanswerable because missed HIV infections are, by definition, unknown. A recent study in London does, however, suggest that for the HIV infections encountered there, anti-HIV assays currently in use give rise to very few false negative results (McAlpine et al., 1995). One thousand and ninety-two confirmed positive sera were examined using twelve current assays and only three false negative reactions were proven. There was the slightest suggestion that assays based on HIV peptides as antigens were less sensitive close to a seroconversion than those based on recombinant antigens; but as other considerations such as technicians' familiarity with the assay, assay compatibility with local testing systems and the importance of maintaining market variety and competition must also be taken into account, the difference, (if any), is not a good enough reason to change established assay usage.

Quality assurance: banishing error

Analysis of known false negative results (there is no means of investigating those that have not been recognised) suggests that laboratory and other human mistakes are at least as important as test sensitivity as sources of error. It is therefore illogical that little effort or attention has been directed to improving quality compared with the large resources that have been applied to improving test sensitivity and the anguished debate that continues on the same subject. In blood transfusion, where quality assurance is better developed than in clinical laboratory practice, instances of erroneous release of HIV infectious donations have been reported from New York State and elsewhere, and releases of HBsAg positive units continue to be reported quite frequently (Linden, 1994). In clinical diagnostic practice, errors are, if anything, more likely. In the UK, investment in laboratory audit is now likely to contribute more to the safety of blood transfusion and the accuracy of clinical diagnosis than further attempts to improve the sensitivity of antibody tests or to incorporate other more expensive tests.

HIV testing of mothers and infants

Although mother-to-infant transmissions of HIV are rare events in the UK, this is certainly not so worldwide. Interest in the diagnosis, management and, where possible, prevention of such infections has now been enlivened by two recent reports. The first suggests that prolonged zidovudine treatment in pregnancy and early life can prevent HIV transmission (Connor et al., 1994) and the second claims that neonatal HIV infection may be reversible (Bryson et al., 1995). The latter finding (of reversible laboratory signs of infections in a single infant) suggests that until, in the face of passive maternal antibody and other immune mechanisms, viraemic dissemination occurs, persistent infection with serological response is not established. This phenomenon of localised infection might not be confined to infants. However, the means of studying early infection using virus culture or PCR in, e.g. adults sexually exposed to HIV, are not readily available. If the single case report of 'reversible' infection in infancy is reproducible it would seem to support the value of zidovudine treatment in the perinatal period. Studies to define precisely when and for how long treatment is needed to protect infants of carrier and acutely infected mothers are now most urgent and should include
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long-term follow up, to allow tests for the delayed appearance of laboratory markers of infection (see below).

The contributions of the diagnostic laboratory to this new opportunity for AIDS prevention will be three-fold. First, the anonymous screening methods that are being used in the UK and elsewhere to test mothers and babies for epidemiological reasons will need, in areas of relatively high prevalence such as London, to be adjusted to allow (with consent), the testing of named mothers. Secondly, as viraemic concentrations in the mothers are probably the chief determinant of transmission to their infants, practical methods are needed to measure them. Thirdly, the inadequacy of present methods for diagnosing HIV in infancy has to be addressed. The difficulty here is not to diagnose the presence of HIV infection but to confirm its absence with certainty. The problem will become more acute if zidovudine is widely used to treat mothers and babies as it may be expected to prevent HIV infection in some cases and delay infection in others. With the present inadequate laboratory means for confirming the absence of HIV infection in babies, who will want to predict successful zidovudine treatment except after what many will regard as an intolerably long interval? Yet the need for prolonged observation of treated babies will be inescapable.

In the wider world the problems of diagnosis and management of 'vertical' HIV transmission are even more intractable. Tests like HIV p24 antigen and PCR are rarely available in developing countries due to their expense and complexity, and although serological tests are usually available they do not distinguish passive from active antibody unless a careful quantitative comparison of anti-HIV titre in specimens collected at intervals is made. Breast feeding also confounds diagnostic testing, a situation not experienced in advanced countries where HIV-infected women rarely breast feed their infants (Mokili et al., 1995).

Home testing for HIV

The US Food and Drug Administration (FDA) is currently considering licensing the first of a number of test systems for HIV diagnosis at home (Bayer, Stryken & Smith, 1995). The current proposal to the FDA is for a home specimen collection system based on self finger-pricking; but other methods, based on saliva collection and its testing in the home by a simple device, will be available soon. Home testing is already possible to measure blood sugar and cholesterol, and to detect chorionic gonadotrophin in pregnancy, and the wish and the ability of anxious and/or curious lay people to conduct tests on themselves at home should not be under-estimated.

The issues that surround these methods are not so much ones of test accuracy (though this may need to be refined), but are concerned with whether psychological and educational support are adequate before and after the test result is known. One issue is the relative value of interactive compared with telephone counselling in supporting individuals who are HIV-positive, and educating the HIV-negative subjects of the test. A second issue is the potential value in personal and public health terms of identifying, by home testing, unrecognised HIV infections and so instigating timely treatment and control measures. Whether home testing is desirable is ultimately a matter of opinion, but in the USA it will soon become a public policy issue and the FDA will have to decide whether or not to sanction it.
Conclusion

After a decade of intense technical development it is often assumed that laboratory tests for HIV (not only for antibody, but also for antigen and amplified HIV gene sequences) are accurate and comprehensively effective. I have sought to indicate why this assumption might be wrong and why more needs to be done to apply available technology effectively to outstanding problems of HIV diagnosis, prevention and management. The maximum benefit will come not from using expensive new technology but from quality assurance and better use of proven serological tests.

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References


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