Review articles

Assays for the detection of recent infections with human immunodeficiency virus type 1

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The Serological Testing Algorithm for Recent HIV Seroconversion (STARHS) is a generic term for several laboratory techniques that can be used to differentiate recent from long standing infections with human immunodeficiency virus-1 (HIV-1). There are several other approaches that identify acute seroconverters, but STARHS methods are distinguished by their ability to identify infections that occurred during an extended period of 4-6 months prior to sampling. While the STARHS techniques have been employed on an individual basis, their main usefulness lies in the potential of estimating the rate of acquisition of new HIV infection, or incidence, in a population by application to cross-sectional sero-surveys. This is substantially simpler and less expensive than cohort studies. As such, STARHS techniques facilitate the timely monitoring of the impact on HIV incidence of factors such as interventions, demographic factors and behavioural patterns.

The major STARHS techniques currently available are described. Furthermore, the principles behind the methods used are discussed and the limitations of the current assays and the confounding factors that may affect assay specificity are described. A model algorithm for the application of a STARHS assay is shown. Finally, we outline recommendations for laboratory quality systems that will improve the efficiency of STARHS testing, reproducibility of results and reliability of incidence estimates.

Introduction

The ability to segregate recently-acquired human immunodeficiency virus type 1 (HIV-1) infections (RHI), i.e. infections acquired in the previous few months, from long-standing ('prevalent') infections is a valuable tool for real-time measurement of the changing patterns of HIV transmission. Although the HIV infection process and the immune response to HIV afford opportunities to recognise recent HIV infection it is only in the last 10 years that these have been exploited to aid the determination of HIV incidence in populations. Differences between individuals present challenges to the application of serological tests of RHI on an individual patient basis.

Virological and Serological Events following infection

The typical evolution of viral and host markers of HIV infection are illustrated in Figure 1. Investigation of virological and serological events that occur during the very early phase of HIV infection indicate that, following local replication in proximity to the inoculation site a high titre viraemia occurs, generally during the second to third week after exposure [1,2]. This allows generalised seeding of the virus in susceptible tissues throughout the body. HIV genomic RNA is present before the patient has developed detectable anti-HIV antibodies and is therefore a powerful marker of recent infection. This phenomenon has been used to identify recent HIV-1 infection by some groups [3-6].

A protein component of the virus core, p24 antigen (p24Ag), is usually detectable [1] within a few days of the onset of viraemia [1]. As the host's immune system initiates a response, levels of both the virus and p24Ag fall. The p24Ag usually becomes undetectable until the degradation of the host immune system associated with progressive HIV-related disease, typically around 10 years later. In most cases HIV RNA remains detectable, albeit usually at levels much lower than in the acute phase. Detection of p24Ag in the absence of anti-HIV antibody may also be used as a marker of recent infection but its presence is unreliable and short-lived (1-2 weeks) and therefore has limited utility for measuring incidence.

The short duration of early p24Ag may in part be explained by it being masked due to complexing with the emerging anti-HIV antibodies. Heat or chemical treatment is able to disassociate antibody-antigen complexes, perhaps allowing extended detection of p24Ag further into anti-HIV seroconversion. However, many individuals with established HIV infection also have complexed

FIGURE 1

Typical evolution of key viral and serological markers during the first weeks following infection with HIV-1 (schematic diagram)
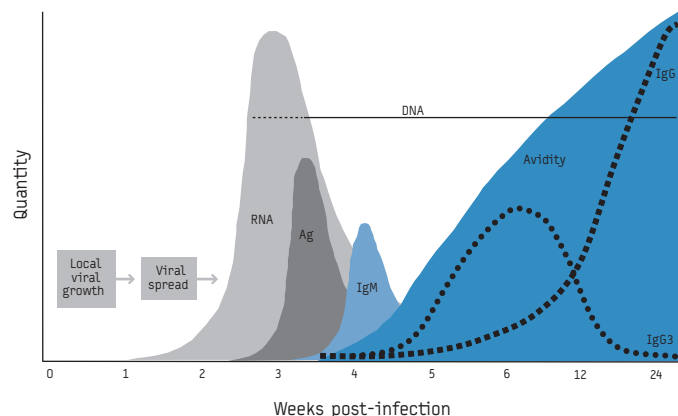
Viral markers: RNA, Ribonucleic acid; DNA, Desoxyribonucleic acid; Ag, Antigen. Immunological markers: IgM/IgG, Immunoglobulin M/G antibodies.

Tests that distinguish recently acquired HIV-1 infection from those that are long-standing take advantage of these events.

p24Ag [7]. Moreover, late in the infection, as the immune system fails, p24Ag is often produced in excess and can in many cases be detected even without the dissociation treatment [8,9]. The presence of p24Ag and/or a high level of HIV RNA after the seroconversion period are usually indicative of rapid disease progression and a poor prognosis [10].

The initial immune response is typically heralded by a virus-specific IgM response [11-13]. This IgM response is variable both in intensity and duration, generally peaking within 1-2 weeks, falling to background levels 1-2 weeks later [14]. Contemporaneously, the long-lived high-titre IgG response develops. A gradual increase in anti-HIV titre occurs over several months and this is the basis of both the 'detuned' and 'BED' assays, discussed later in the context of the Serological Testing Algorithm for Recent HIV Seroconversion (STARHS).

Standard HIV screening and diagnostic assays

Standard commercial screening and confirmatory tests are mostly unable to distinguish between long-standing and recently-acquired infections. When specimens are taken during the short period, typically no more than 2-4 weeks, between onset of seroconversion and attainment of the maximum signal in the conventional diagnostic tests, it may be relatively straightforward to diagnose an incident infection on the basis of the rapidly evolving serological pattern. However, to be confident, a combination of supplemental tests needs to be done which may include some or all of the following: Immunoblot (Western blot/line immunoassay); and assays for the detection of: HIV RNA; p24Ag; and IgM anti-HIV. The Western blot assay involves the detection of antibodies against specific HIV-1 proteins separated by molecular weight. The presence and relative reactivity of each specific antibody can be identified, and a pattern typical of recent seroconversion may be recognised. A potential hazard of utilising limited Western blot patterns (i.e. reactivity with few HIV-1 proteins) as evidence of RHI, particularly during the earliest phase of anti-HIV seroconversion, is the significant risk of confusing non-specific reactions with HIV seroconversion. Furthermore, the interval during which this approach may be used, perhaps 3-4 weeks after infection, is too short to permit reliable measurement of HIV incidence on realistic population sizes.

The Serological Testing Algorithm for HIV Seroconversion (STARHS)

The typically rapid immunological response to HIV infection means that within less than a month of anti-HIV seroconversion commencing, standard HIV test kits are unable to distinguish recent from long-standing infections. However, a number of adapted or novel techniques have been developed that are able to identify recent infection over a longer time frame than that achievable with conventional assays. These methods are intended to be applied to individual specimens in which the presence of anti-HIV-1 antibody has already been confirmed, and the approach is known generically as the Serological Testing Algorithm for Recent HIV Seroconversion (STARHS).

The STARHS approach offers a number of important advantages over other methods for determining HIV-1 incidence. Unlike cohort studies which require repeated testing of individuals, and where results may be biased by people leaving the study, STARHS testing can be carried out retrospectively on stored single specimens from cross-sectional sero-surveys. In comparison with cohort studies, applying the STARHS approach is cheaper, quicker and simpler to perform. Furthermore, STARHS testing can be performed on a real-time basis thus allowing a measure of recent infection at the time of a study as opposed to incidence derived from a cohort study which cannot be ascertained until after the follow-up sample has been collected and tested.

STARHS/RHI Window Period

The STARHS technique allows HIV-1 incidence to be determined from representative panels of stored anti-HIV-1-positive specimens gathered over a given period from a particular population whose size is known. The duration of the period between seroconversion in the original (sensitive) HIV-1 screening assay and conversion (from recent to long-standing) in the STARHS method must be well-defined and typically in the order of several months, and is critical to a STARHS assay being able to furnish a population incidence rate (Figure 2). The duration of this STARHS window needs to be determined carefully, and this requires panels of specimens from individuals whose date of seroconversion is known or closely approximated. Modelling these data allows the relationship between time since seroconversion and the expected average signal in the STARHS method to be described mathematically. From this, and additional data on known long-standing (>12 months) infections, the chosen cut-point, dividing recent from long-standing, may be set such that it provides an appropriate balance of sensitivity and specificity, and this is typically associated with a mean RHI window in the region of 3-6 months. The duration of the STARHS window is limited by the effects of individual variation on antibody titre and rate of antibody production and maturation. The longer the time after infection, the more pronounced these individual differences become, leading to increasing misclassification [15,16]. Although

Principles underpinning the serological testing algorithm for recent HIV infection (STARHS)
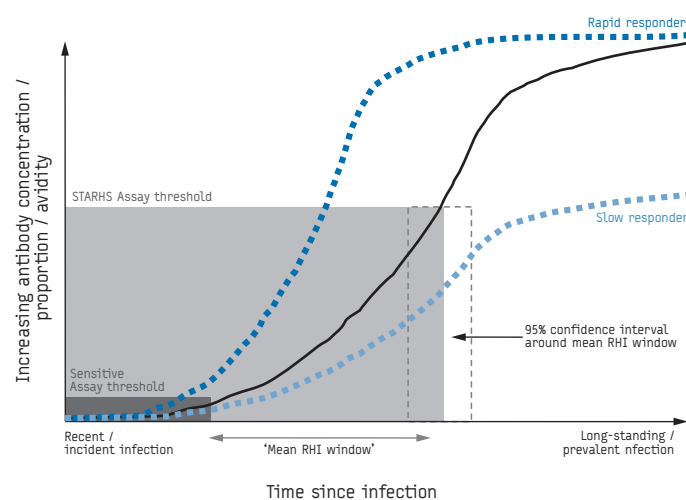
The STARHS approaches are applied to confirmed anti-HIV-1-positive specimens and, with the exception of the IgG3 and Inno-LIA approaches, rely on the marker employed, e.g. avidity, increasing over the first several months after seroconversion is detected by a sensitive screening method. If a test specimen gives a result below a pre-determined cut-point, it is deemed to have been a recently acquired HIV-1 infection (RHI). The cut-point is set such that it provides an appropriate balance of sensitivity and specificity, and this is typically associated with a RHI window in the region of 3-6 months. However, the uncertainties around this model should be considered, including the accuracy of the RHI window (95% confidence intervals) and the person-to-person variability shown in the diagram as outliers with either a 'rapid' or a 'slow' response. The former may appear to have a long-standing infection some time sooner than the average RHI window, and the latter may appear to be an RHI some for a considerably longer time.

of lesser magnitude, differences in seroconversion sensitivity between anti-HIV-1 screening tests employed in STARHS should also be taken into account, particularly when moving between generations of screening tests, for which the difference could be more than two weeks [17].

Definition of the STARHS window permits measurements of HIV-1 incidence to be made on achievable populations, but their robustness will depend on several factors, not least the accuracy of the mean STARHS window period employed. When applying STARHS on an individual (diagnostic) basis, the duration of the STARHS window period cannot be accurately defined, and arguably need not be. Importantly, it must be borne in mind that the STARHS windows described represent the mean interval between the earliest time at which an HIV-1 diagnosis may be made and conversion to long-standing status in the STARHS assay, and not the upper limit. This is derived by examining specimens from many seroconverting individuals in whom the immune response will mature differently. Accordingly, taking the 155 days' window advised for the BED-CEIA assay (described below), a substantial proportion (roughly one-half) of those infected will already have converted in that assay to a long-standing infection at under 155 days since seroconversion, and the remainder at over 155 days; very few will actually convert on day 155. The consequence of this, when applied to individuals, is that some are likely to be advised inaccurately that, in the former example, their infection is over 155 days-old and therefore long-standing, and in the latter, that it was under 155 days-old and therefore classified as recent.

Furthermore, STARHS results consistent with an RHI are known to arise and be persistent in a small proportion of those infected for years and in those presenting late in the course of infection [18], as discussed below. Because the rate of misclassification as an RHI is a key variable influencing the accuracy of population incidence estimates this is coming under closer scrutiny, and has led to proposed correction factors for incidence estimation [19,20]. However, these do not provide a means to ensure an error-free finding when applying STARHS individually. Nevertheless, an improved understanding of sensitivity, specificity and predictive values associated with STARHS testing should provide an appropriate platform for providing advice, care and public health action on an individual basis. It may be advisable when using STARHS as a diagnostic indicator to communicate the timing of infection less definitively, e.g. when the result is consistent with a RHI: 'The findings suggest HIV may have been acquired in the last 12 months'.

Assays for recent HIV-1 infection
A number of assays can be used within a STARHS programme (Table).

The 'detuned' assay
The 'detuned' assay was the first assay to be described as being able to identify specimens from individuals recently infected with HIV-1 for the purposes of incidence calculation. Employing the recommended assay cut-off, the technique recognises HIV-1 seroconversions that have occurred on average four to six months prior to collection of the positive specimen [15,16,21]. However, the period during which recent infection can be identified can be altered by changing the cut-off applied to the assay. The method relies on the generalisation that anti-HIV titres in the plasma rise gradually, and at a similar rate in each infected individual, over a period of several months following seroconversion.

The 'detuned' approach takes confirmed anti-HIV-1-positive specimens and re-tests them with an enzyme immunoassay (EIA) that has been made less sensitive ('detuned') by increasing the dilution at which each specimen is tested from 1/76 to 1/20,000 and by reducing the incubation times. Although assay variability is partially accommodated by the inclusion of a calibrator, obtaining accurate results by the detuned approach is technically demanding, requiring precise preparation of high serum dilutions and strict adherence to incubation conditions. Recent seroconversion is inferred if the confirmed anti-HIV-1-positive specimen is negative in the less sensitive EIA.

The 'detuned' approach has been described for two different immunoassays: the Abbott HIVAB 3A11 (Abbott laboratories, United Kingdom (UK)) and the bioMérieux Vironostika HIV-1 microelisa (bioMérieux, UK). Production of both assays has now ceased with the last lot of bioMérieux Vironostika assays expiring in summer 2008. Both these assays use a semi-purified viral lysate antigen adsorbed to the solid phase. In both cases the viral lysate derives from an isolate of the subtype B strain of HIV-1. The Abbott HIVAB 3A11 antigen is also 'spiked' with purified native gp41 antigen. The use of an antigen from a single HIV subtype means that

TABLE

Methods described which may be employed on serum/plasma specimens in serological testing algorithm for recent HIV seroconversion (STARHS) applications to distinguish recent from long-standing HIV-1 infection

STARHS method	Type	Principle	Reference
Abbott HAVAB (3A11)	Modified commercial (withdrawn 2003)	'Detuned' – standard assay, sensitivity reduced to extend seroconversion window	[15]
Abbott AxSYM HIV 1/2 gO	Modified commercial	Avidity of anti-HIV antibodies	[31]
Calypte BED EIA	Commercial	Proportion of total antibodies that are HIV-specific	[23]
bioMérieux Vironostika HIV-1 microELISA	Modified commercial (withdrawn 2008)	'Detuned' – standard assay, sensitivity reduced to extend seroconversion window	[16]
IgG3 anti-HIV	In-house	Transient presence of IgG3 isotype antibodies against HIV p24Ag	[36]
IDE-V3 EIA	In-house	Reactivity with two selected HIV antigens is used to predict likelihood of recent infection	[34]
Inno-LIA HIV	Modified commercial	Relationship of reactivity with various HIV antigens	[37]
Ortho Vitros *ECi* anti-HIV 1+2	Modified commercial	Avidity of anti-HIV antibodies	[33]
Particle agglutination (SeroDIA-HIV)	Modified commercial	'Detuned' – standard assay, sensitivity reduced to extend seroconversion window	[38]

heterologous antibodies (i.e. those formed against viral subtypes not utilised in the assay) may not bind the antigen as effectively as the homologous antibody. This generally causes an increase in the period during which the assay would determine a specimen to be from an RHI [22]. Reactivity in the 'detuned' assay is standardised against a calibrator specimen to give a standardised optical density (SOD), thus smoothing out run-to-run variability.

BED-CEIA assay

The BED-CEIA (capture enzyme immunoassay) is a commercial product (Calypte Biomedical, United States (US)) designed specifically for the purpose of identifying infections that were acquired recently [23]. Being a class-specific IgG antibody capture EIA, it differs in its mechanism from the 'detuned' assays as its reactivity is dependent, not on the absolute titre of HIV-1-specific antibody, but on what proportion of all the IgG captured from an HIV-infected person's serum is directed against the HIV antigens employed. In early infection, the proportion of HIV-specific antibody is lower than in a long-standing infection. As the BED-CEIA does not directly measure the amount of antibody present it is technically more robust than the 'detuned' techniques, the principle on which it is based being more forgiving about the accuracy of dilution of test specimens, incubation times and temperatures.

The BED-CEIA was designed to overcome some of the subtype differences associated with the 'detuned' assays, utilising a trimeric branched peptide. Each branch comprises a synthetic oligopeptide derived from the immunodominant region of the transmembrane gp41 glycoprotein of HIV-1 subtype B, CRF_01 AE and subtype D, hence the assay name 'BED'. These three peptides were selected to cover much of the breadth of antigenic diversity, in theory allowing a single window period to be used with the BED-CEIA test, whatever the infecting HIV-1 subtype. However, it has been shown that differences in window periods between subtypes do occur in the BED assay, though perhaps less pronounced than in the detuned assays (see below).

Avidity Assays

A further approach to identifying recent infection is to investigate the maturity of the HIV antibody response by investigating its avidity. Antibodies of low avidity are usually indicative of recent infection and this approach has been shown to be valid for many viral infections [24-26]. Although avidity assays have previously been described for use with HIV-1 [27-30], it was not until recently that assays that could be used for HIV-1 incidence determination were described [31-33].

The method described by Suligoi et al. is a modification of the third generation anti-HIV-1/-2 assay that runs on the Abbott AxSYM random access analyzer, and is therefore easy to perform. It uses a method whereby the specimen is pre-incubated with the chaotropic agent guanidine (guanidine hydrochloride) [31,32]. Guanidine treatment of the specimen primarily disrupts the hydrogen bonds that help determine the secondary structure of the antibody, although it may also have a residual effect on the subsequent antibody-antigen interaction. The treatment has a greater effect on early antibodies, the active site of which has a less defined structure and can be degraded by mild denaturation so that they are less able to bind their homologous antigen, thus reducing the signal. As the antibody response matures, the active site becomes increasingly resistant to disruption.

When assessing the avidity of an antibody response, the level of signal obtained after chaotropic treatment is compared with the signal produced when pre-incubating the specimen in a neutral diluent such as phosphate buffered saline (PBS). When the antibody is highly avid and therefore largely resistant to the chaotrope, the two signals in the immunoassay will be very similar. The binding of early, less avid, antibodies on the other hand will be much reduced when treated with the chaotrope, and this will produce a reduced signal compared to the untreated aliquot. The RHI window for the AxSYM avidity assay has not yet been determined precisely, but it is thought to be close to six months.

Recently, an alternative antibody avidity assay has been described that also uses guanidine but runs on the Vitros analyzer (Ortho Diagnostics, UK). It has an RHI window of approximately 142 days when employing a threshold avidity index of 80% [33]. Currently no published data exist on the widescale application of these avidity assays, and work is continuing to refine their performance characteristics and the window period, particularly for HIV-1 non-B subtypes.

IDE-V3 assay

The IDE-V3 immunoassay is based on two conserved highly immunogenic epitopes found in the envelope glycoproteins of HIV-1 [34]. One is derived from the immunodominant epitope (hence 'IDE') of the transmembrane glycoprotein gp41; the second derives from the V3 loop of the outer glycoprotein gp120. The IDE antigen comprises two consensus oligopeptides of 30 amino acids, one from HIV-1 group M and one from subtype D. The V3 component comprises a blend of five oligopeptides derived from the HIV-1 subtypes A, B, C, D and CRF_01 AE. The IDE-V3 assay is not available as a commercial kit, but can be assembled by the user from basic ingredients that are available commercially.

Technically the assay is structured as a simple indirect enzyme-immunoassay, employing a 96-well microplate format, with the 8-well columns alternately coated with the IDE and V3 oligopeptides. A dilution of each specimen is tested against both the IDE and V3 antigens. In its current format this assay has to be assembled by the user from individual components and, although its principle is relatively straightforward, its wider availability as a robust STARHS approach awaits further standardisation of the reagents and controls.

To discriminate recent from long-standing infection this assay employs a mathematical formula which draws on reactivity of the specimen with the antigens from each region. The formula was derived from testing panels of specimens known to be from either recent (<6 months) or long-standing infections. Although the authors imply that the assay is able to identify recent infections that date back no more than six months, this appears to have been based on polarised specimen sets: specimens representing RHI, which had mostly been collected soon after seroconversion, and specimens representing long-standing infections, many of which may had been collected considerably later than six months following seroconversion. Consequently, the continuous relationship between the assay output and time since seroconversion has not been mathematically modeled, and the exact duration of the RHI window period has yet to be calibrated.

Sakarovitch et al., applying STARHS assays to seroconverting individuals in Cote d'Ivoire found that the IDE-V3 assay, while having good specificity (96.3%), had poor sensitivity (42.3%), and

this suggests strongly that its seroconversion window is likely to be considerably shorter than six months [35]. Currently this assay is being used as part of the French national screening programme to determine the proportion of newly diagnosed HIV infections that were recently acquired. Work is continuing to improve the estimation of the RHI window period for this assay.

Other STARHS approaches

A number of other approaches have been described that distinguish recent from long-standing HIV-1 infection. These include:

IgG3 Anti-HIV: It is known that the IgG isotypes formed in response to an infection may vary during the course of an infection. Research investigating the IgG isotype response to a range of HIV-1 antigens using a Western blot approach identified that isotype IgG3 was usually present transiently during the first few months of HIV-1 infection [36]. The investigators found the antigen against which the IgG3 response was most reliable was p24. These findings were converted into a simple EIA based procedure whereby IgG3 to p24Ag is typically detectable for only the first 1-4 months of infection. Unfortunately, however, this method has not yet been translated into a commercial kit.

Inno-LIA HIV Adaptation: The Inno-LIA™ HIV I/II Score is a line immunoassay, similar to a Western blot but employing only a limited selection of synthetic oligopeptides and recombinant antigens of HIV-1 and HIV-2. Its routine application is as a confirmatory test to investigate whether screen-reactive specimens are true or false. For the STARHS application the intensity of each band in the Inno-LIA test is read using a slightly modified scoring system. An algorithm is applied to the scores which allows the segregation of the results into recent or long-standing HIV infection [37]. The approach is expensive, but may have utility where it is already routinely employed as the confirmatory diagnostic test.

Several other approaches have been described, including one based on a particle agglutination test in a 'detuned' format [38] and an oral fluid assay [39], but neither of these assays has been applied on a large scale and the RHI window periods have not been established.

New STARHS approaches are under development and should be expected to become available over the next few years.

Limitations of STARHS assays

The accuracy of STARHS assays is affected by a number of factors that are likely to be encountered when testing populations of HIV-infected individuals, and these are outlined below:

Infecting HIV subtype

The detuned STARHS methods have been based on the use of HIV-1 clade B antigens. Because the immunodominant epitopes differ between HIV-1 clades it is likely that the heterologous antibody responses may show lower binding affinities and that this, in turn, could alter the RHI window period, in most cases extending it. Should this be the case, HIV incidence would be over-estimated unless the RHI window is adjusted. This presents serious difficulties when dealing with epidemics of mixed clades, such as are now established in at least some European Union countries.

Studies on populations infected with non-B viruses have indeed revealed that the period during which an infection is identified as recent is significantly different to that for clade B infections. For example, employing an SOD threshold of 1.0 in the Vironostika detuned assay the average RHI window is 170 days, whereas for the CRF_01 AE virus it is 356 days [22] and for clade C it is 360 days [40]. Comprehensive findings are not available on this issue, and few are actually published.

As discussed above, the BED-CEIA method was designed to overcome problems associated with the lower affinity of heterologous antibody responses by employing a multimeric antigen representing much of the antigenic diversity associated with the immunodominant region of gp41. The manufacturer's product insert for the BED-CEIA advises the use of a single mean RHI window period of 155 days [41]. However, studies have demonstrated that the mean RHI window period for clade C is substantially longer, at 181 days, and for CRF_01 AE it is much shorter, at 115 days [42]. The impact on more recently described methods like the avidity, IDE-V3 and IgG3 methods is as yet unknown.

Acquired immunodeficiency symdrome (AIDS)/Low CD4 Count

The failing immune system associated with advanced HIV disease has long been known to be associated with a decline in anti-HIV antibody levels [8], and this would be expected to impact the specificity of those STARHS methods that depend primarily on the quantification of antibody. Indeed, misclassification rates for the detuned methods have been published, and for Vironostika it has been estimated that approximately 5% of AIDS cases will be misclassified as a recent infection [16]. For the BED-CEIA approach, the AIDS misclassification rate has been estimated at 2-3% [41]. Misclassification of AIDS cases by the IDE-V3 assays is approximately 9% [34]. On the other hand, as the avidity of antibody binding is not related to the quantity of antibodies, it would be expected not to be similarly affected, and preliminary evidence suggests this may be so.

Antiretroviral Therapy

It has been observed that combination anti-retroviral therapy (ART) leads to misclassification of long-standing infections as recent. The exact mechanism has not been elucidated, but simplistically, it is likely that the ART suppresses viral replication to such a degree that the chronic stimulus to the humoral immune response is removed, leading to a decline in anti-HIV antibody titre. The effect is most pronounced during the first few months after ART initiation (authors' unpublished findings). However, in comparison to the very high anti-HIV titres typically found in HIV-infected individuals this effect is modest and would not be sufficient to render state-of-the-art HIV screening tests negative.

Other Confounders

In some cases there is no clear common factor associated with a misclassification by STARHS. In an extensive study among HIV-1-infected men who have sex with men in a UK city, several long-term infected individuals with naturally suppressed viraemia (<50 copies/ml) were flagged as a recent infection by the detuned assay [18]. There is some evidence that the BED-CEIA approach misclassifies a substantial minority of long-standing infections as recent and consequently leads to inflated incidence rates [19,20,43].

Quality Control Measures

As with any laboratory diagnostic method STARHS assays must be performed within an appropriate quality system. This includes the documentation of processes, use of standard operating procedures, appropriate training of staff and evidence of competency. In the authors' experience, the type and condition of equipment can significantly impact on the transferability of STARHS methods

between laboratories and lead to inconsistent results. Several of the key elements are discussed below:
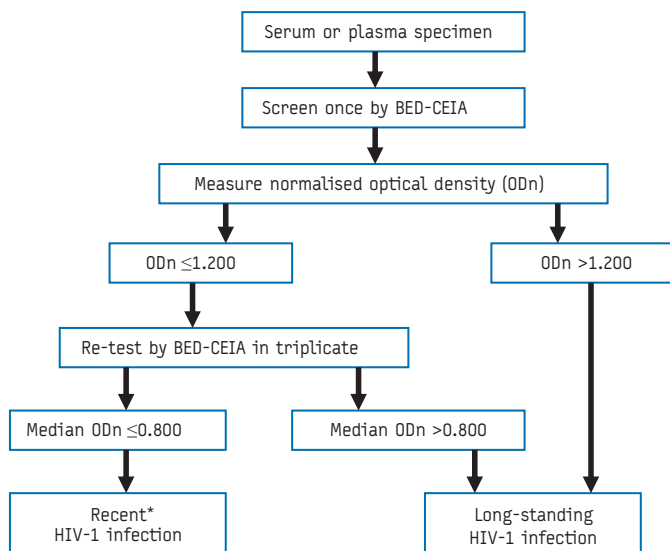
Robust and Reliable Methods

With the exception of the BED-CEIA the methods currently available are either modifications of commercial kits, or 'in-house' assays. Whichever sort of STARHS method is employed, it is important to select an assay that suits the laboratory's resources and skills and the population to which it is to be applied. The method should be capable of providing findings of acceptable accuracy and reproducibility. The use of modified or 'self-assembly' techniques is more vulnerable to inconsistency of performance and in those circumstances validated production and quality control processes must be in place to verify consistency of performance.

Confirmatory Algorithms

In common with other diagnostic methods, the results of a STARHS assay will show some variability. To improve the reliability of the test result, the well-established methods ('detuned'; BED-CEIA) include an algorithm of triplicate retesting of specimens whose reactivity is in the range associated with recent acquisition, and a defined margin above, e.g. for the BED-CEIA a normalised optical density (ODn) of up to 1.200 (Figure 3). However, while this improves the accuracy of the STARHS measurement it does not identify the samples misclassified due to the factors discussed above such as advanced HIV disease. Similar approaches need to be developed for the other STARHS techniques.

Assay Calibrators and Assay Controls

A common approach to smoothing out lot-to-lot and run-to-run variation in performance is to employ one or more calibrator specimens which would show reactivity in the mid-range. They are employed to adjust the signal obtained with each test specimen against the reactivity of the calibrator, and thus control variations over time. At present, only the 'detuned' and BED assays incorporate a calibrator, generating respectively a 'standardised optical density' (SOD) and a 'normalised optical density' (ODn). In addition, other controls are normally included (e.g. non-reactive; long-standing). Even when all controls are supplied as part of a commercial STARHS kit it is best practice to include further controls of expected reactivity, either from a third party supplier or produced by the user laboratory, to provide the means to monitor assay performance independent of the kit manufacturer. Such controls provide a tool to ensure the assay is performing within expected parameters, and provide the basis for acceptance or rejection of each set of results.

External Performance/Quality Assessment (EPA/EQA)

An important component of ensuring laboratories' performance is adequate is the blinded examination of small panels (typically 4-8 members) of specimens of unknown status. Such schemes require significant investment to establish and maintain. At present, EPA/EQA schemes exist only for the 'detuned' and BED assays. Furthermore, there is arguably a need for larger panels to qualify laboratories embarking on the application of STARHS methods.

Concluding Remarks

A wide range of STARHS approaches have been described and new methods are under development. They clearly have a potentially important role both in public health monitoring and individual diagnosis. The evidence indicates that the current methods are generally able to distinguish recent from long-standing HIV-1 infections. However, the rigors of assigning an accurate duration to the interval between infection or seroconversion and the time at which the transition to a long-standing infection is assigned by STARHS remain challenging. This is due to the diversity both of the host immune response and of the antigenicity of HIV-1. When applying the method as an epidemiological tool to estimate incidence these variables may be controlled if there is a single prevalent HIV-1 subtype and its associated mean window is accurately known. In many parts of Europe, however, the HIV-1 epidemic is already heterogeneous. The BED-CEIA was designed to accommodate this, but despite this it has emerged that the mean RHI window, even for the small number of clades for which it has been derived, ranges from 115 to 181 days and this alone could lead to over- or under-estimates of incidence of approximately 50% [42].

It remains to be seen whether the assays currently being developed will provide improved accommodation of HIV-1 diversity. The complexities of the multiple variables involved in designing broadly applicable STARHS methods, optimising them, calibrating their performance and recognising their limitations present enormous challenges. A global initiative led by the WHO/UNAIDS has been created which is pooling the experience and resources of laboratory scientists, epidemiologists and statisticians working in the STARHS field. We should therefore expect improved STARHS methods and applications to emerge over the next few years.

FIGURE 3

Example of the STARHS testing process, employing the BED-CEIA procedure

* The duration of the recent HIV infection window that is advised in the BED product insert is 155 days. This is the mean duration, which is an important value when estimating population incidence rates. It is not the upper limit of the STARHS window. Consequently, when interpreting STARHS findings on an individual basis it must be borne in mind that a substantial proportion of those whose ODn is ≤0.800 will actually have been infected more than 155 days earlier. Similarly, some whose ODn is >0.800 will have been infected less than 155 days earlier. As one might expect, findings in close proximity to the cut-point of 0.800 are more likely to be a misclassification.

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