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New Approaches For Detecting Recent HIV-1 Infection

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Abstract

Detecting newly HIV-infected people has gained much attention recently for extending the usefulness of HIV testing and surveillance in providing information about HIV incidence, monitoring transmission of uncommon subtypes or drug resistance, and examining possible clinical implications for infected individuals. Recent developments in our ability to detect and distinguish recent and long-term HIV-1 infection using laboratory tests have made the detection of new infections realistic and practical. Sensitive/less-sensitive testing strategy provided a simple laboratory tool (3A11-LS, the detuned assay) to detect recent seroconversion in a cross-sectional population. This approach, termed "Serologic Testing Algorithm for Recent HIV Seroconversion" (STARHS), is based on differential antibody titers in recent versus long-term infections. Additional approaches that rely on different principles and properties of the evolving anti-HIV antibody response, such as antigen or epitope specific antibodies, antibody titers to specific antigen, proportion of HIV-IgG, antibody affinity and conformation dependence of antibodies, are further being investigated. Irrespective of the approach used, our data suggest that an assay that uses antigen(s) derived from a single HIV subtype is likely to have subtype-specific performance. Antigens derived from multiple subtypes will be needed to achieve similar performance among different subtypes. Using a branched gp41 peptide from subtypes B, E, and D, we have recently developed an IgG-capture BED-EIA that detects an increasing proportion of HIV-IgG in total IgG following seroconversion and can be used to detect recent infection. This 96-well EIA has several advantages over previous approaches and should be widely applicable worldwide. The quantitative nature of these assays requires stringent performance criteria that include calibration and quality control reagents. Ongoing research in this area will further enhance our understanding and may expand the use of this approach to alternative specimen types, such as oral fluids and dried blood spots.

Key words

Recent HIV-1 infection. Incidence. Transmission. Diagnosis.

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Why Recent HIV-1 Infection?

The HIV pandemic continues to spread with about 40 million people infected worldwide by December 2001. Approximately 5 million new infections are believed to have occurred in the year 2000 alone[1,2], corresponding to 15,000 new infections per day. In the United States, in spite of public health initiatives about 40,000 new infections per year continue to occur. Further reductions in new infections will require coordinated prevention efforts targeted to groups with highest incidence. The Centers for Disease Control and Prevention's (CDC) stated goal of reducing new infections in the U.S. by 50% in five years will require a comprehensive approach, a key element of which will be identifying populations with high incidence. Similarly, worldwide prevention efforts will need to focus on new infections to make a major impact on the spread of the epidemic. The changes in HIV prevalence may or may not reflect trends in incidence, indicating the importance of continued monitoring for incidence[3-28]. Moreover, studies directed at identifying HIV-1 subtypes[28,29] or patterns of drug resistance in newly infected persons can provide important information about the direction and dynamics of the epidemic[30-37]. Incidence is also important for identifying and selecting appropriate cohorts in preparation for vaccine trials[7,8,25,28,38-47].

Detection of recent infection may have clinical relevance at the individual level as well. Identifying individuals during the early phase of infection can lead to early treatment[48,49], which in turn may reduce the establishment of tissue virus reservoirs and improve long-term prognosis[36,48,50-57]. Early treatment, coupled with appropriate counseling and partner notification, can help to reduce secondary transmission from recently infected individuals to their sex partners[4,58-60].

Although current HIV antibody testing methods detect infections, they do not distinguish between recent (incident) and long-term (prevalent) infections. Seropositives in any population include both these groups but are indistinguishable by traditional diagnostic methods (Fig. 1). A number of approaches have been used to identify recent infections and estimate HIV-1 incidence. It is to be noted that most new laboratory approaches are for investigational use only and may be best suited for population incidence studies for public health initiatives. More data is required for their application in individual cases.

Detecting Recent HIV Infections

Acute Retroviral Syndrome

Clinicians often diagnose early HIV infections based on symptoms, termed as "acute retroviral syndrome"[49,61-66]. This early diagnosis is often confirmed by the presence of HIV-1 RNA or p24 antigen (see later). Sensitivity and specificity of this approach in detecting new infection will vary among clinicians. Although useful in clinical practice, it does not have practical applications in public health settings. Benefit to the individual of early treatment may result in better prognosis but the "flu-like" symptoms are not unique to HIV infection, and therefore, a laboratory confirmation would be highly desirable.

Follow-up and Record Based Studies

The traditional approach for detecting incident infections has been the longitudinal monitoring of seronegative people for seroconversion (Fig. 2)[10,16,18,29,67,68]. This requires recruitment of a large number of seronegative volunteers. Such studies involve repeated sample collection and testing of these individuals at set intervals[10,16,18,56,69-71]. By design, these studies are cumbersome, expensive, may have a recruitment bias, and may not be timely. Risk reduction and behavior change as a result of counseling during recruitment may yield a lower incidence than the actual rate occurring in the cohort population. Further, unavoidable loss to follow-up may confound the analysis.

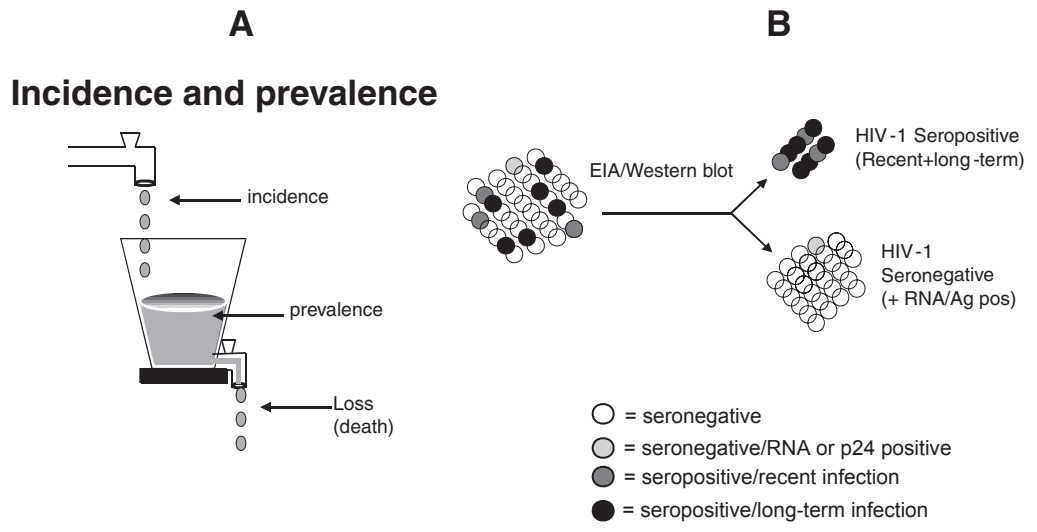

Figure 1. *A: Schematic showing incidence (recent infection) and prevalence in a population. B: Diagnostic tests identify seropositive specimens which include both recent and long-term infections.*

Another approach is record-based retrospective studies, which examine test results on individuals who repeatedly visit certain sites for specific purposes[9,20,72-75]. For example, blood banks with a large pool of repeat donors can examine the frequency of seropositivity, with a prior negative result indicating a new infection, yielding an incidence estimate in this low risk population[67]. Similar strategies have been used to determine incidence among sexually-transmitted diseases (STD) clinic attendees or young homosexual men[18,76,77].

Laboratory-Based Methods

Laboratory-based methods that identify recent HIV infection would allow incidence measurements. Such tests could be applied to any set of specimens, including stored cross-sectional specimens. The natural history of HIV infection indicates that the way various parameters evolve may help in distinguishing early from later infection (Fig. 3). Tests that can detect or quantify these parameters can be applied to detect recent infection.

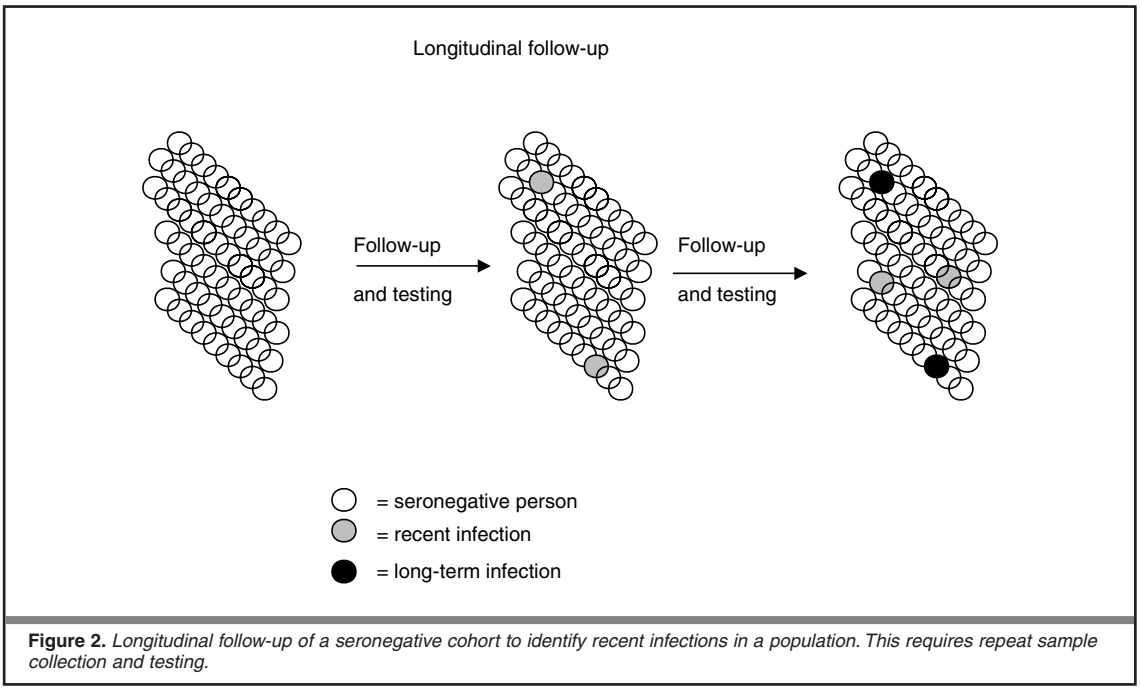

Figure 2. *Longitudinal follow-up of a seronegative cohort to identify recent infections in a population. This requires repeat sample collection and testing.*

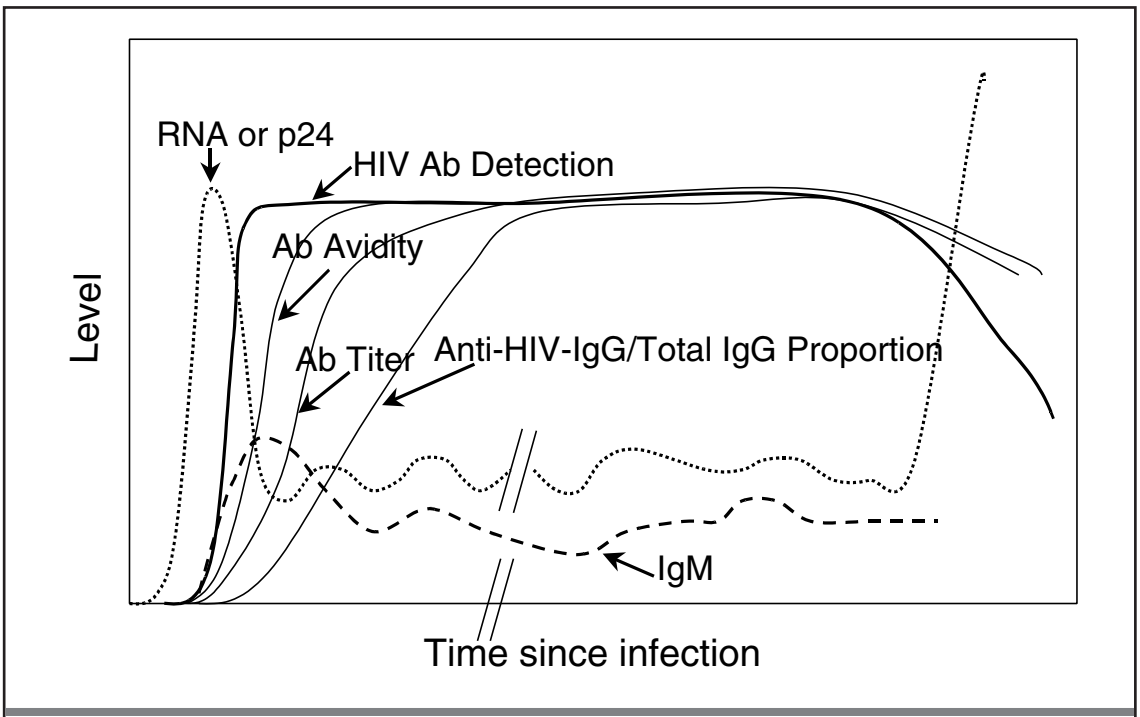

Figure 3. *Schematic depicting changes in various parameters that define early and late HIV-1 infection.*

a) HIV-1 RNA or p24 among antibody negative people

Definitive detection of HIV-1 RNA or p24 antigen in the absence of specific antibody (Ab) indicates primary HIV infection (PHI). These methods are useful and have been applied in some studies to estimate incidence[21,26,28,48,49,55-57,59,62,71,78-91]. However, the duration of this status (RNA/p24 positivity - Ab negativity) is quite short (~1-2 weeks), with antibodies appearing soon thereafter. The short duration makes it difficult to capture enough people in this phase of PHI to render an incidence estimate with a reasonable confidence interval, especially when the incidence is less than 5% per year. Moreover, this approach requires testing a large number of seronegative people (at risk of infection) repeatedly for the presence of RNA or p24 antigen. Detection of these analytes is significantly more expensive and technically more complex than detecting HIV antibody. In general, a test applied to the seropositive population would require less testing and be more cost-effective.

b) Sensitive/less-sensitive testing strategy

In 1998, Janssen, et al.[92] described a modified, less-sensitive enzyme immunoassay (EIA) which, when used to test HIV positive specimens, detected recent HIV seroconversion. The assay was made less sensitive by using a higher serum dilution (1/20,000 compared to 1/400) and reduced incubation times (Fig. 4). The strategy was based on the fact that antibody titers increase following seroconversion. The sensitive EIA (3A11) plateaus soon after seroconversion, but the less-sensitive EIA (3A11-LS) had a longer dynamic range, and standardized optical density (SOD) levels indirectly reflected antibody titers. A predefined cutoff distinguishes specimens with antibody titers lower than 1/20,000 (recent infection) from those with titers higher than 1/20,000 (long-term infection). Those classified as recent infections were estimated to have seroconverted within the last 129 days (seroconversion duration, 95% CI, 109-149). Due to the quantitative nature of this assay, a careful calibration was required to ensure reproducibility. A cali-

A

Comparison of 3A11 and 3A11-LS

PARAMETER	3A11	3A11-LS
Specimen Dilution	1/400	1/20,000
Specimen Incubation	60 min	30 min
Conjugate Incubation	120 min	30 min
Classification	Based on assay cut-off	Based on calibrator

B

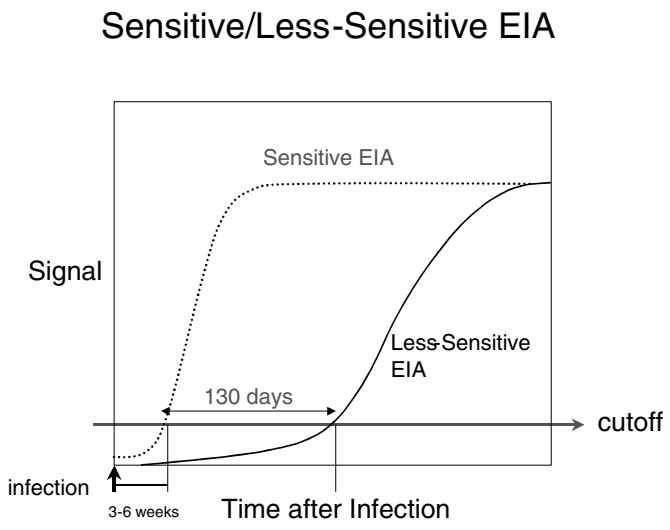

Figure 4. *A: Comparison of sensitive (3A11) and less-sensitive (3A11-LS) EIAs. B: Sensitive EIA plateaus soon after seroconversion, while less-sensitive EIA has longer dynamic range and takes about an additional 130 days to register reactive.*

brator reagent and additional controls were developed to monitor the assay. A schematic showing application of the 3A11-LS assay, as a second test, to classify seropositive specimens as recent or long-term infection is shown in figure 5. The application of this modified EIA for the detecting incident infection has generated tremendous interest in the United States and elsewhere[3,4,29,44,92-96]. However, recent work demonstrated that the assay had different performance characteristics among people infected with subtype E in Thailand[29], with a seroconversion duration of 270 days. This necessitated a change in seroconversion duration and/or cutoff values to make it applicable to areas of the world with multiple HIV-1 subtypes. In addition, the viruses would have to be subtyped from cases of recent infections and their percent distribution among prevalent cases would have to be known. These are complex tasks in most settings. Use of subtype B antigens, typical of most commercial EIAs, in the 3A11 assay appear to result in subtype-specific bias. These data indicated that newer approaches should incorporate antigens derived from multiple HIV-1 subtypes to get similar reactivity in people infected with different subtypes. Additional shortcomings of the 3A11-LS assay include specimen dilution of 1/20,000, which was cumbersome and contributed to high variability, and the requirement of dedicated equipment to perform the assay. Further, the 3A11 assay is an early generation EIA and there have been problems of production and availability. Another assay, the Organon Teknika less sensitive assay (OT-LS) has been recently adopted to replace the 3A11-LS[97]. Again, because this assay uses antigens derived from a single subtype (B), it also has subtype-specific bias and different performance when used on subtype E specimens (CDC, unpublished data). The use of the assay with other subtypes is currently being investigated.

c) Other Approaches

HIV-specific antibodies are usually detectable within 3-4 weeks after infection and form the basis of serologic diagnosis. Qualitative and quantitative changes occur in the antibody population following seroconversion. These changes relate to antibody isotype, antibody avidity/affinity, antibody titer (see above), conformation dependence of antibodies, proportion of HIV-IgG, and antigen or epitope specificity. These properties can be exploited to distinguish early from long-term HIV infection with variable success[98]. Like less-sensitive assays, these approaches require testing of only those specimens confirmed to be seropositive, in contrast to detecting HIV-1 RNA/p24 among antibody negative people. Both less-sensitive assays indirectly measure antibody titers to multiple viral proteins. Antibody titers to individual proteins were further examined to assess which specific protein(s) may be the most useful in distinguishing specimens from recent and long-term infections[98]. Envelope proteins, specifically gp41 and its oligomers (gp120/160), were found to be the most useful. The immunodominant peptide of gp41 resulted in differential titers and can be easily used in an EIA.

With regards to isotypes, IgM antibodies are detectable early but often the duration and intensity of this response is quite variable[86,89,99-105]. An early study indicated occasional detection of IgM in patients with long-term infection, which may result from periodic viremia and antigenic stimulation[102]. This makes detection of specific IgM an unreliable marker of recent HIV infection.

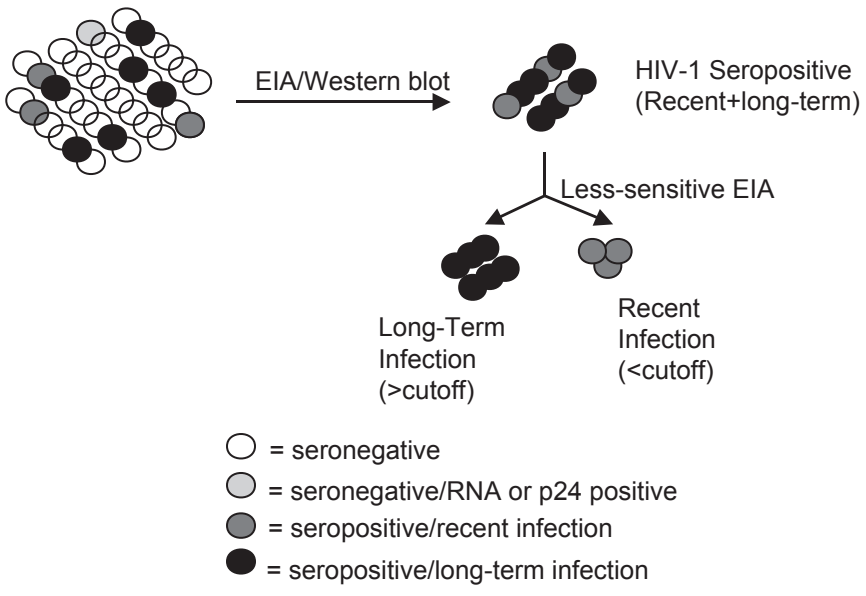

Figure 5. *The use of sensitive/less-sensitive testing strategy to detect recent HIV seroconversion.*

Antibodies to various HIV proteins and epitopes are elicited at different rates[99,106-112]. Anti-*gag* (p24) and anti-*env* (gp41) responses are observed early in infection while antibodies to *pol* proteins develop later in infection. However, p24 antibodies decline with the development of clinical AIDS. Within envelope proteins, robust antibodies to gp41 immunodominant epitopes develop early, while antibodies to the V3 loop portion of gp120 develop later in infection. A strategy of detecting the level of antibodies to two or more different proteins or peptides representing early and late infection may provide clues to the timing of infection. Such an approach would require testing specimens by at least two tests based on two antigens. Although this represents an interesting approach, further testing of longitudinal specimens from seroconvertors would be needed to study the kinetics of immune responses to specific proteins and to assess the efficacy of this methodology.

The increase in antibody avidity and affinity is a hallmark of antibody maturation following seroconversion[69,98,104,113-120]. Although traditional methods to assess antibody affinity are cumbersome, inclusion of a chaotropic agent to dissociate low avidity antibodies in an enzyme immunoassay (EIA) can provide avidity information in a simple assay. Such approaches using an additional incubation with chaotropic agents (e.g. Urea, KSCN, NH4SCN) or low pH buffer, in conjunction with commercial EIAs, have been used in the past[98,120] to detect recent infection with variable success. Detecting urea-susceptible antibodies or observing thermal elution of low affinity gp41 peptide antibodies demonstrated relatively simple methods to detect recent infections[98]. A careful examination of evolving antibody affinity to various HIV antigens, subtype homologous and heterologous, may provide a basis for the development of new assays using affinity parameters. Such studies are in progress.

Antibodies to conformational epitopes are observed earlier than to those directed to linear antigenic sites[98,113,114]. A comparative detection of antibodies using native and denatured antigen may provide clues regarding timing of infection, thus forming a basis for detecting recent infection. Antibodies to heat denatured or reduced/alkylated gp120 did permit some separation of recent and long-term infections, but there appeared to be significant overlap[98].

d) IgG-Capture BED-EIA

Another parameter that increases following seroconversion is the proportion of anti-HIV-IgG (in total IgG) present in serum. EIAs that use antigen-coated wells detect HIV antibodies with high sensitivity but are not quantitative and plateau soon after seroconversion. A competitive IgG-Capture EIA was devised to detect an increasing proportion of HIV-IgG in the serum following seroconversion (Fig. 6). This assay captures HIV and non-HIV IgG in the same proportion that is present in serum. HIV-IgG was then detected by a branched-gp41 peptide antigen, incorporating sequences from subtypes B, E, and D. Interestingly, an increase in the OD values

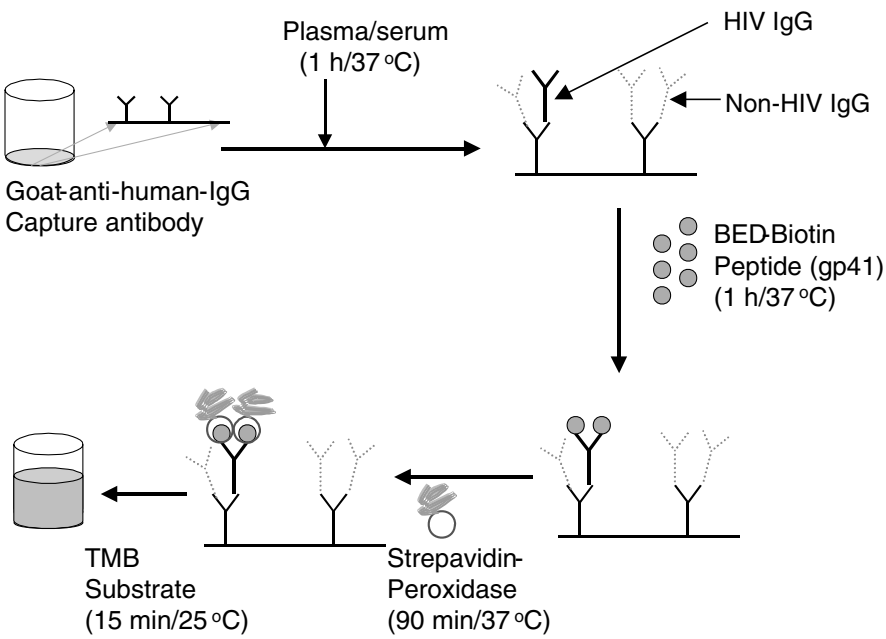

Figure 6. *Schematic showing various assay steps of the IgG-Capture BED-EIA that measures increasing proportion of HIV-IgG in serum following seroconversion.*

was observed for more than 2 years after seroconversion, indicating that such an assay may have a useful dynamic range for distinguishing recent from long-term infection[121,122]. Application of this approach in longitudinal specimens from a large number of seroconvertors established criteria that defined a cutoff (normalized OD [OD-n] of 1.0) and seroconversion duration (160 days) resulting in an optimal combination of sensitivity and specificity to detect recent infection. Antigen derived from multiple subtypes permitted detection of recent infection among different subtypes with similar dynamics. Further validation in specific subtype subsets or in specimens not used for optimization demonstrated that the observed number of incident specimens was within 10% of the expected number[98,121,122]. However, since the assay is based on the proportion of HIV-IgG present in total IgG, the differences in IgG concentration in various populations may affect the assay. For example, if normal IgG concentration ranges are higher among Africans compared to Caucasians, seroconversion duration may be somewhat longer in African population, assuming the kinetics of HIV antibody synthesis are similar. Therefore, further validation of this assay using longitudinal specimens from seroconvertors from different regions would be important to address this issue.

This assay has several advantages over previous approaches based on antibody titer. Its 96-well format allows the use of generic washers and readers. The assay is performed using a specimen dilution of 1/100, compared to the 1/20,000 dilution required by less-sensitive approaches. Moreover, the assay is not significantly affected by variation in dilution, as long as the proportion of HIV/non-HIV IgG remains the same. A calibrator is used to normalize the OD values, and quality control reagents help monitor the assay runs. The inter-run and inter-operator reproducibility has been excellent (unpublished data).

Our experience suggests that quantitative assays measuring specific parameters (e.g., titer, antibody proportion, antibody avidity or affinity etc.) to differentiate early from late infection will require stringent quality control criteria and a careful monitoring of each run to ensure high precision and reproducibility. These assays differ significantly from qualitative assays that detect HIV antibodies for diagnostic purposes and for which a mere yes/no answer would suffice.

Misclassification of Patients with AIDS and Other Co-infections

Using methods that rely on various properties of HIV antibodies, such as titer, proportion, and possibly affinity, may inadvertently result in misclassification of a small fraction of patients with clinical AIDS as recently infected[92,97,121,122]. Declining immune function during AIDS, coupled with high viral antigens which can combine with antibody to effectively reduce its availability for titer, proportion or affinity measurements may contribute to this misclassification. About 2-5% of AIDS patients were misclassified as recent infections with both the 3A11-LS and IgG-Capture BED-EIA. However, limiting testing of those who are known not to have AIDS would reduce its impact on incidence estimate measurements.

Since the IgG-Capture BED-EIA is dependent upon the increasing proportion of HIV-IgG in relation to total IgG present in the serum, conditions or co-infections that elevate total IgG (hypergammaglobulinemia) may result in false high incidence. Initial studies among those with HIV and TB did not indicate high rate of misclassification. Further studies are warranted among those with possible hypergammaglobulinemia.

Incidence Calculation and Interpretation

Incidence per 100 persons per year is calculated by using an appropriate formula that accounts for the total number of people at risk (seronegative + recent infections) and corrected for the year using the factor (365/T), where T is the seroconversion duration[92,121]. This requires that information about the number of people tested for HIV-1 infection, the number who are found to be antibody negative and the number who are seroincident be available for incidence estimates. The use of these approaches should remain limited to population incidence estimates for public health initiatives and prevention activities because of the potential for misclassification.

However, these assays may have applications at the individual level as well, because early treatment of newly infected people may have clinical implications. Once these assays have achieved wider use for incidence estimates, they could be evaluated for individual use but with a more conservative criteria. For example, specificity of detecting recent infection can be increased using a lower cutoff[121]. To avoid misclassification, other clinical parameters such as CD4 levels and disease stage should also be considered.

Future Prospects

A number of approaches can be used to further improve the current assays. Towards that goal, developing a novel chimeric recombinant protein, with antigenic portions derived from divergent subtypes, in a single molecule can be of great utility. Our data have shown that such an antigen should be used to achieve an equivalent quantitative signal among various subtypes, irrespective of the assay principle. We are in the process of developing such recombinant molecules for incorporation into new generation assays.

It is also likely that two or more principles (e.g., proportion or titer of HIV-IgG and antibody avidity) can be combined into a single assay to further improve our ability to distinguish recent and long-term infections. Our study using a proportionate assay with a urea dissociation step demonstrated that seroconversion duration increased to 180 days

(from 160 days without urea), but this did not enhance overall sensitivity and specificity[121]. Nevertheless, approaches that combine titer and antibody avidity may be worth further investigation. Assays that quantify changes in titer or proportion of specific IgG subclasses may prove to be more valuable than overall changes in IgG.

HIV rapid test protocols may be modified to detect recent HIV-1 infection (Granade and Parekh, unpublished data). However, since the rapid tests are interpreted visually, the modifications require very careful calibration, evaluation, and validation. Such a development can have implications in areas of the world where significant testing is routinely done by rapid HIV tests.

Diagnostic assays have been modified to detect HIV antibodies in dried blood spot (DBS)[123,124] and oral fluid (OF) specimens[125,126]. These alternative specimens can be used with sensitivity and specificity equivalent to serum or plasma. Similarly, it is possible that the tests to detect recent infection may also be adapted for use with DBS or OF specimens. However, this would require a parallel study of matched serum/DBS or OF specimens. Because of the quantitative nature of these assays, collection procedures will have to be highly standardized since inconsistency in the collection process could have affect the level of detectable antibody.

Conclusions

Recent developments in our ability to detect recent infections by testing cross-sectional prevalent positive specimens are proving to be very valuable. Efforts are underway to generate HIV incidence data in various risk groups in the United States and in other countries. This should help to identify groups with highest incidence so that resources can be appropriately targeted, and the effectiveness of prevention efforts assessed. However, these new tests for incidence estimates have not been implemented in most African and Asian countries with very high prevalence and incidence. This is due, in part, to a lack of resources as well as to variable test performance of less-sensitive assays with the specimens of subtypes prevalent in those countries. A simple assay that performs similarly for specimens of different subtypes should be very useful in identifying new infections for incidence estimates worldwide and may prove to be a valuable tool in overall HIV prevention efforts.

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