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# How can we better identify early HIV infections?

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## Abstract

**Purpose of review—**Detection of early HIV infections (EHIs), including acute HIV infection (AHI), is important for individual health, prevention of HIV transmission, and measurement of HIV incidence. We describe markers of EHI, diagnostic strategies for detecting these markers, and ways to incorporate these strategies into diagnostic and HIV incidence algorithms.

**Recent findings—**For individual diagnosis in the United States and Europe, laboratory-based diagnostic algorithms increasingly incorporate fourth-generation HIV antigen tests, allowing for earlier detection. In some sub-Saharan African settings, symptom-based screening is being explored to identify subsets of persons at high risk for AHI. Point-of-care diagnostics designed for AHI detection are in the pipeline and, if validated, represent an opportunity for real-time AHI diagnosis. At the population level, multiassay algorithms are promising new strategies for estimating HIV incidence on the basis of several assays applied to cross-sectional samples. These algorithms can be developed to optimize performance, in addition to cost and logistical considerations.

**Summary—**There are important recent advances in detection of EHIs at the individual and population levels. Applying optimal combinations of tests in diagnostic and HIV incidence algorithms is urgently needed to support the multiple goals derived from enhanced detection and discrimination of EHIs.

### Keywords

acute HIV infection; early HIV infection; fourth generation; multiassay algorithm; point of care

## Introduction

Early HIV infection (EHI) is the period from HIV acquisition through the establishment of chronic infection. EHI encompasses the eclipse phase (when HIV is not detectable in the

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plasma) [1], acute HIV infection (AHI) (when HIV is detectable, but the antibody response is not), and recent infection (when HIV antibodies are detectable by sensitive diagnostic assays, but the immune response is immature). Together these phases last approximately 3–6 months. EHI ends when a stable viral 'set point' is established, a key determinant of subsequent disease progression [2] and chronic transmissibility. Directed laboratory testing can locate individuals within this spectrum. One common system proposed in 2003 by Fiebig *et al*. [1] defined six EHI stages on the basis of the maturation of viremia and antibody responses using then-current diagnostics. With substantial advances in EHI diagnostics, efforts are now underway to improve on this staging system [3••].

At the individual level, EHI detection is important for proper diagnosis. When EHI clinical manifestations occur, they are almost universally misdiagnosed as another endemic illness, such as malaria or influenza, resulting in inappropriate subsequent testing and treatment [4,5••,6]. More rarely, severe manifestations of EHI, such as meningitis, encephalitis, or esophagitis, can occur, which must be distinguished from both non-HIV causes and manifestations of AIDS.

EHI detection is also important for infection management. Detecting and correctly staging EHI enables rapid initiation of treatment with potential for long-term clinical benefits [7,8•]. Progressive changes occur over the first few weeks of infection: irreversible depletion of CD4 lymphocytes in the gut [9], mucosal damage allowing gastrointestinal bacterial products access to the circulation [10], replication in the central nervous system [11•], and the establishment of latent HIV reservoirs, which to date has rendered HIV incurable [7,12]. Initiation of antiretroviral therapy (ART) during EHI improves host immune control of viral replication [13,14•,15] and has been associated with improvements in CD4 cell counts over time [16•,17], reduced systemic inflammation [18], preserved cognitive function [19•], and a reduced latent reservoir [8•]. In the United States, immediate treatment for persons with EHI has been recommended [20•].

Detection of EHI is also important for HIV prevention. Rapid viral replication results in elevated viral load in blood and genital secretions, making the host extremely infectious. Infectiousness is amplified during EHI by viral variants that are more infectious than viruses from the same individual later in infection [7]. Because of increased infectiousness during EHI, most mathematical models suggest that EHI is responsible for a disproportionate share of HIV transmissions [21–24]. Accordingly, ART during EHI can be expected to make the host less infectious [13,14•,15]. Detection of EHI may also facilitate behavior change, as some persons who become aware that they are HIV-infected, including those with AHI, report declines in unprotected sex [25•,26,27].

Finally, for HIV surveillance, discriminating EHI from established infection can be used to estimate HIV incidence. Information from such surveys is becoming increasingly important for measuring impact of prevention interventions in cluster randomized trials [28•] and for monitoring the impact of biomedical interventions in populations as they are brought to scale.

In this review, we discuss the markers of EHI, strategies for detecting these markers, and how these strategies have been combined into algorithms for individual EHI case detection and HIV incidence estimation.

## Clinical Markers of Acute HIV Infection

During the first several weeks of HIV infection, many persons display an acute retroviral syndrome with symptoms, such as fever, diarrhea, and rashes [29]. Because these symptoms are nonspecific, they cannot reliably identify AHI independently. Recently, three studies of outpatient clinic populations in Uganda, Mozambique, and Kenya have reported results of targeted AHI testing based on patients presenting with fever or suspected malaria [4,5••,6]; all three reported high AHI prevalence confused with more common diseases. In the recent Kenyan study, fever was just as likely to be AHI, as it was to be malaria [5••]. These studies demonstrate that far from being a rare event, AHI is likely a common clinical syndrome in HIV endemic parts of sub-Saharan Africa. These results have yet to translate into clinical practice or guidance recommending widespread AHI testing in general medical settings in sub-Saharan Africa [30•,31•]. However, this may change with improved, cheaper AHI diagnostics that are now becoming available.

Symptoms, along with behavioral or demographic characteristics, can also be used to identify a subset of persons more likely to have AHI, who then receive laboratory screening. For instance, a risk score developed for AHI in Malawi reduced the proportion of sexually transmitted disease clinic attendees who needed laboratory screening by 60%, but identified 95% of persons with detectable AHI [32]. Targeted, symptom-based screening has been replicated in emergency departments and outpatient facilities in developed and developing countries, with a wide range of risk factors being predictive of AHI [5••,33•,34•,35].

## Detection of Acute Viremia (Preantibody Phase)

HIV RNA and p24 antigen, components of circulating virions in plasma, are detectable before HIV antibodies are formed (Fig. 1). HIV RNA appears first, approximately 7–10 days after HIV infection, and can be detected using PCR or other nucleic acid amplification technologies (NAATs). Currently, RNA testing is performed in well resourced laboratories. Samples can be tested individually, or multiple samples can be tested at once in ‘pools,’ with only samples from positive pools subsequently tested individually [36,37]. Optimal pooling strategies for different conditions have been described [38] with substantial reductions in cost, but with increases in time and complexity.

The next biomarker to appear is p24 antigen, which is detectable approximately 5–7 days after HIV RNA. P24 can be detected by ‘fourth-generation’, antigen-antibody ‘combo’ assays with very good performance characteristics [39•,40,41]. Fourth-generation laboratory immunoassays have become routine in European countries and have replaced HIV antibody-only tests for HIV screening in many US laboratories [42]. Unfortunately, neither fourth-generation laboratory immunoassays nor traditional HIV NAATs are suitable for rapid, point-of-care diagnosis in the clinic settings in which AHI is common and the potential for efficient AHI case-finding the greatest.

Notably, two first-generation rapid NAAT devices for use in AHI diagnosis have been introduced quite recently. The Alere q HIV-1/2 Detect (Alere Technologies, Jena, Germany) is designed for both point-of-care early infant diagnosis and potentially adult AHI diagnosis. The first assessment shows excellent performance characteristics in infants [43•], but field test results for adult AHI are not available. The SAMBA HIV semiquantitative point-of-care test was developed primarily for point-of-care viral load monitoring, and secondarily to detect persons with AHI, but to date it has not been evaluated for AHI either [44•].

Previous efforts to deploy rapid tests for AHI have been mixed: the Alere Determine HIV-1/2 Ag/Ab is a lateral flow-based rapid test designed to detect and differentiate established HIV infection (through detection of HIV antibodies) and EHI (through detection of p24 antigen) at the point of care using plasma, serum, or whole blood. The antibody portion of the test has performed exceptionally well, detecting antibodies sooner than other rapid tests, but the antigen portion has not. In clinic-based evaluations on whole blood in Malawi, France, the United Kingdom, Australia, and South Africa, the p24 antigen portion of the assay failed to detect any of the AHI cases [45–48,49•,50]. Sensitivity for detection of p24 varied widely in laboratory assessments of stored serum and plasma with the p24 well displaying lower sensitivity than other fourth-generation assays [51•–54•,55–59]. These concerns notwithstanding, the test has provisional US FDA approval for use in clinical laboratories with quality assurance programs [60]. Development and field testing of a reliable point-of-care assay for AHI detection remains an important goal that merits investment.

## Detecting Postantibody Early HIV Infection

Approximately 1 week after the appearance of p24, immunoglobulin M antibodies are detectable through third-generation immunoassays, several weeks earlier than first or second-generation immunoassays. Although third-generation assays detect HIV earlier, they classify very few persons with EHI when used in combination with fourth-generation assays, due to the very brief ‘window period.’ Identifying persons with EHI in the postseroconversion period is also important, as it allows for brief early interventions for treatment or prevention.

In the past, results from supplemental Western blot tests extended the EHI period by several weeks, and could be used to stage persons in the early postseroconversion period [61]. However, the Western blot is no longer being performed in most clinical laboratories. Among confirmatory tests replacing the Western blot are the INNO-LIA HIV I/II Score (Innogenetics, Gent, Belgium) [62] and a newer rapid test, the Bio-Rad™ Geenius HIV 1/2 Assay (Bio-Rad Laboratories, Inc.). These diagnostics provide similar semiquantitative information and can distinguish EHI from long-standing infections [55,63•] if such information is sought. Updated staging systems, which rely on diagnostics currently in use, are urgently needed.

Additional assays rely on the evolving nature of the antibody response following HIV acquisition with respect to quantity, proportion, avidity, or isotope. These antibody-testing approaches, referred to as ‘incidence assays’ because of their use in HIV surveillance, have

been developed to classify EHI among antibody-positive individuals. These include the serologic testing algorithm for recent seroconversion, the BED capture enzyme immunoassay (BED-CEIA), and limiting-antigen (LAg) avidity assays (discussed in more detail below).

Genetic information can also be used to stage EHI. Within a single host, the HIV genome is typically homogenous following acquisition and diversifies over time into complex quasispecies, making viral diversity another potential measure of EHI duration. Some of these methods include 'molecular clock' models [64], high-resolution melting technologies implemented manually or through automated platforms [65–67,68••], HIV sequencing techniques [69], and Pairwise Alignment Positional Nucleotide Counting, a novel computational method [70••,71]. These assays are currently used for research purposes and some have been incorporated into HIV incidence estimation activities.

## Evolving Practice in Early HIV Infection Detection and Staging

In 2014, nearly all well resourced countries have adopted fourth-generation immunoassays as first-line screening tests for HIV, on a variety of automated and manual platforms. In 2014, the Centers for Disease Control and Prevention (CDC) issued a new laboratory algorithm for use in the United States with a sequence of US FDA-approved HIV assays designed to detect persons in either EHI or chronic HIV infection, and to differentiate HIV-1 from HIV-2 [72,73,74••] (Fig. 2). The algorithm begins with a highly sensitive fourth-generation test, and, for reactive samples, an immunoglobulin G immunoassay capable of differentiating HIV-1 from HIV-2. Antibody-nonreactive specimens can be tested individually for HIV-1 RNA to identify patients who were recently infected. Use of fourth-generation and NAAT technology has increased detection of AHI in US settings where this has been implemented [36,75,76•–79•].

In many European countries, including the United Kingdom, France, and Italy, modified 'incidence assays' are used routinely to complete the staging of new infections as being in EHI versus long-standing HIV infection. Assay results are provided to guide patient management, in addition to the information they contribute to surveillance. In the United States, negative, indeterminate, or evolving results of supplemental antibody tests are often used to make the diagnosis of EHI, but the use of supplemental incidence assays has not been embraced. In 2014, however, the US CDC issued a new surveillance case definition, which, for the first time, includes EHI as a reportable category referred to as 'stage 0' HIV infection [80••]. This guideline, along with the arrival of new supplemental assays capable of EHI staging, may lead to more postseroconversion EHI diagnosis in the United States.

## Evolving Practice in HIV Incidence Estimation

HIV incidence estimation, which also relies on the detection of EHI, can be measured directly in cohorts of HIV-uninfected persons followed longitudinally for HIV seroconversion. However, longitudinal methods require large cohorts, followed at frequent intervals, over long periods with minimal, nondifferential loss to follow-up [81]. Additionally, the process of frequent HIV testing can itself affect HIV acquisition risk [82].

Back-projection techniques were developed for incidence estimation in surveillance data, using assumptions about the timing between HIV acquisition and HIV testing. However, due to inevitable time lags, these techniques were inadequate for measuring recent trends, and estimates were often imprecise due to assumptions about the duration between infection and diagnosis [83–86].

Cross-sectional HIV incidence estimation can measure recent HIV incidence trends with one or more biomarker at a single time point. The Serologic Testing Algorithm for Recent Seroconversion relies on the maturity of the antibody response [87,88], but variability between HIV subtypes makes this approach unreliable [89,90]. The BED-CEIA was designed to detect multiple HIV-1 subtypes and differentiate recent from long-standing seroconversion based on the proportion of anti-HIV immunoglobulin G, but misclassifies persons with advanced disease and immunosuppression as having EHI, leading to overestimates [91–94]. The newer LAg assay measures the avidity of antibody binding to low concentrations of a multisubtype peptide derived from an immunodominant region of gp41 [95,96]. However, this assay suffers from similar challenges of misclassifying persons with advanced HIV disease, elite controllers, and persons with viral suppression and partial seroreversion following ART as having EHI [96,97••].

The key recent advance in cross-sectional HIV incidence estimation is the development, refinement, and validation of multiassay algorithms (MAAs) [28•,98••,99•,100•,101••, 102,103•,104]. MAAs apply a sequential, hierarchical set of assays to classify confirmed seropositive samples into recent versus long-standing infection categories. MAAs first screen in persons with possible EHI, then screen out those with risk factors for false-recent misclassification, such as ARV-treated individuals and elite controllers. As with the effort to develop clinical uses of EHI tests, the central challenge remains reducing false-recent results without over-shortening the window period [105].

MAAs are evolving rapidly and large-scale efforts to systematically evaluate the full range of new EHI tests are now underway [106••]. To determine the optimal MAA for each setting, many possible algorithms are systematically applied to the same group of samples in different orders and with a range of cut-points [101••,103•]. For example, in the United States, primarily a subtype B epidemic, the optimal MAA consisted of CD4 more than 200 cells/$\mu$l, BED-CEIA optical density less than 1.0, avidity less than 80%, and HIV viral load more than 400 copies/ml [101••]. A significant milestone in the MAA concept occurred with NIMH Project Accept (HPTN 043), which used an MAA as its primary outcome measure [28•]. This MAA used the same tests, but in a different sequence with different cut-points: BED-CEIA optical density less than 1.2, avidity index less than 90%, CD4 cell count more than 200 cells/$\mu$l, and viral load more than 400 copies/ml [28•]. MAAs can also be developed to meet specific cost or logistical constraints. For example, MAAs using high-resolution melting technology, rather than CD4 count, can be conducted entirely on stored serum or plasma [68••]. Still others, such as those using the LAg assay, can be performed on stored serum or plasma, collected via dried blood spots, and performed with fewer assays [97••].

## Conclusion

Careful study of the natural history of EHI has resulted in an array of clinical, virologic, immunologic, and genetic markers that can be used to identify EHI cases and classify them into different stages. The brevity of each stage and the difficulty to identify them and distinguish them from established HIV infection at the point of care remain major challenges. Nonetheless, substantial progress has been achieved at the individual and population levels. As more persons with prevalent HIV infection learn their HIV status, a larger share of those in need of HIV diagnosis will be those with EHI, making its detection increasingly important. Ever better strategies for simple, efficient detection of EHI and measurement of HIV incidence are critical public health priorities.

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•• of outstanding interest

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**Key Points**

- In the United States and Europe, laboratory-based diagnostic algorithms increasingly incorporate fourth-generation HIV antigen tests, allowing for earlier HIV detection.
- In some sub-Saharan African settings, symptom-based screening has been explored to identify subsets of persons at high risk for AHI.
- Point-of-care diagnostics designed for AHI detection are in the pipeline and, if validated, represent an exciting opportunity for diagnosis in real time.
- MAAs are a promising new strategy for estimating HIV incidence at the population level.

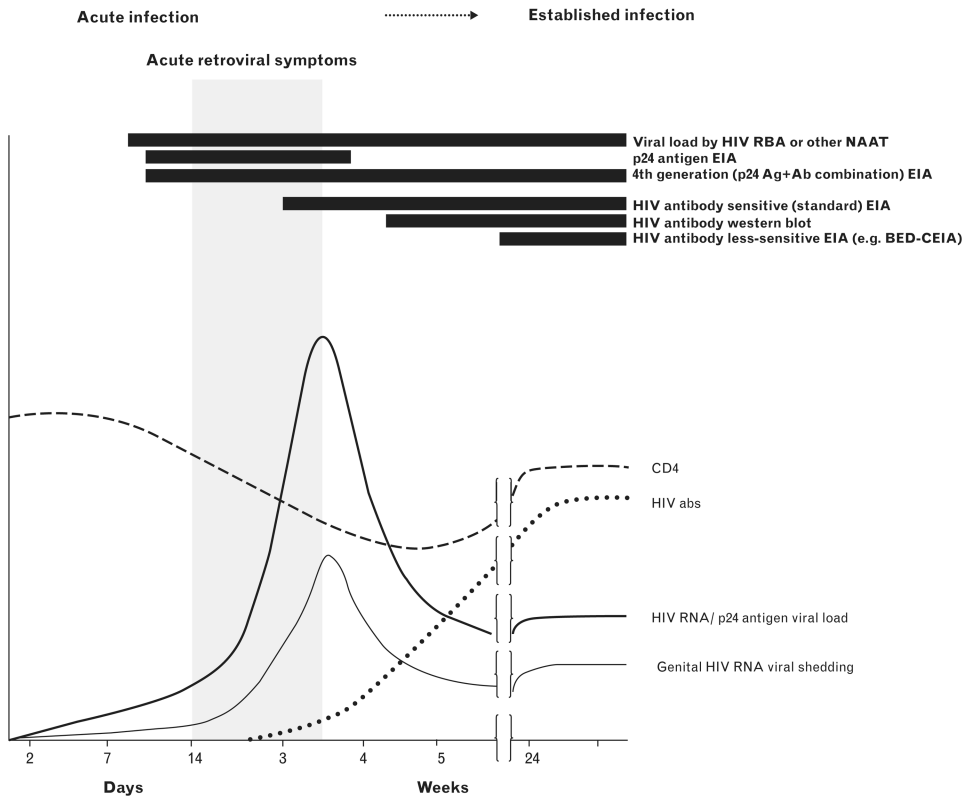


**Figure 1.**

Timing of early HIV infection biomarkers and symptoms. This figure depicts the trajectories of CD4 count, the appearance of HIV antibodies and symptoms, and blood and genital shedding soon after HIV infection. Black bars indicate timing of detection. Reproduced from [35].

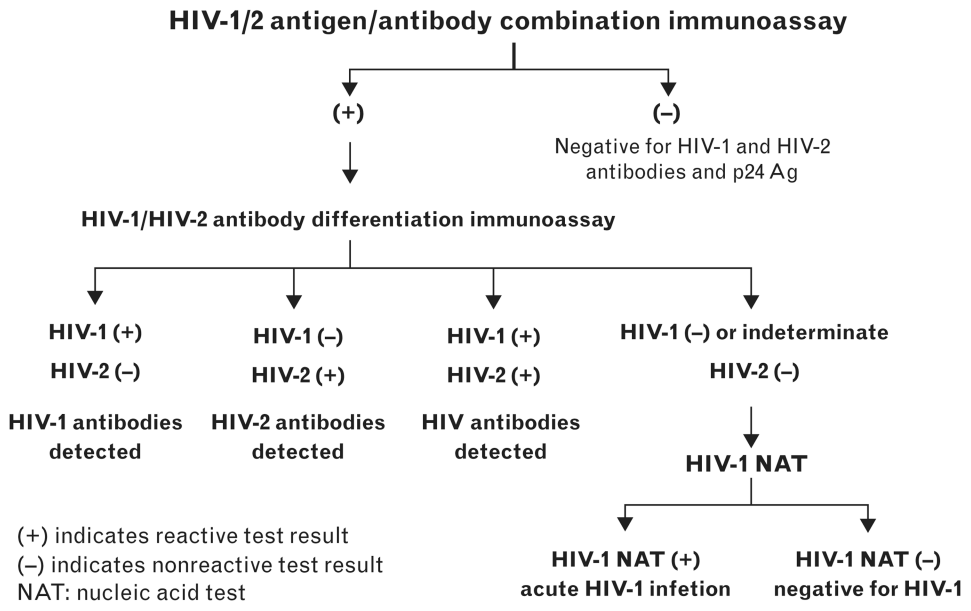


**Figure 2.**

Updated testing algorithm for US HIV diagnosis. This figure depicts the algorithm issued by the Centers for Disease Control and Prevention for HIV diagnostic testing in the United States in 2014. It distinguishes HIV-1 from HIV-2 and acute HIV infection from established HIV infection. Reproduced from [74••].