SUPPLEMENT ARTICLE

The Detection of Acute HIV Infection

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Acute human immunodeficiency virus (HIV) infection (AHI) can be defined as the time from HIV acquisition until seroconversion. Incident HIV infection is less well defined but comprises the time from the acquisition of HIV (acute infection) through seroconversion (early or primary HIV infection) and the following months until infection has been well established, as characterized by a stable HIV viral load (viral load set point) and evolution of antibodies with increased concentration and affinity for HIV antigens. During AHI, a viral latent pool reservoir develops, the immune system suffers irreparable damage, and the infected (often unsuspecting) host may be most contagious. It has proved very difficult to find individuals with AHI either in longitudinal cohorts of subjects at high risk for acquiring the virus or through cross-sectional screening, and the opportunity for diagnosis is generally missed during this phase. We review the technical strategies for identifying individuals with acute or incident HIV infection. We conclude that further technical advances are essential to allow more widespread detection of patients with AHI and to affect HIV treatment outcomes and transmission prevention.

Acute human immunodeficiency virus (HIV) infection (AHI) has attracted tremendous attention because of the importance of this stage of infection to virtually all aspects of HIV epidemiology and biology. First, it is clear that the study of individuals with AHI offers the best opportunity for understanding the HIV transmission event in humans [1]. All other data about HIV transmission come from studies of rhesus macaques [2, 3], and these data complement but cannot substitute for data from acutely infected humans. Second, it may be possible to intervene during AHI to limit HIV viral replication and integration into a latent pool that renders HIV incurable [4, 5]. Third, subjects with acute infection are maximally contagious [6, 7], because of either high viral load, unique and as yet unexplained viral properties, or lack of any binding antibodies [8]. Importantly, HIV acquisition, and therefore AHI, is not uncommon in pregnant women [9, 10], which probably increases the risk of vertical transmission of HIV [11].

Early diagnosis during the critical stages of AHI represents a tremendous opportunity for treatment and prevention interventions. After acquisition of HIV, a series of events follows that is characterized by different patterns of viral antigens and antibody responses and that can be used to diagnose HIV infection. These evolving patterns have implications for the detection of early infection and will be reviewed.

STAGES OF EARLY HIV INFECTION

In the macaque model, simian immunodeficiency syndrome can be detected in ⩾1 receptive cell within 3 days after sexual exposure [3]. Based on the fact that viral sequences in the earliest stages of infection in heterosexual subjects are extremely homogenous [12], it can be concluded that either a single virion particle or very small number of them are transmitted from a diverse number of quasispecies in the exposure inoculum (see below). These results demonstrate an unexplained "transmission bottleneck." After transmission there is an initial "eclipse phase" (Figure 1 and

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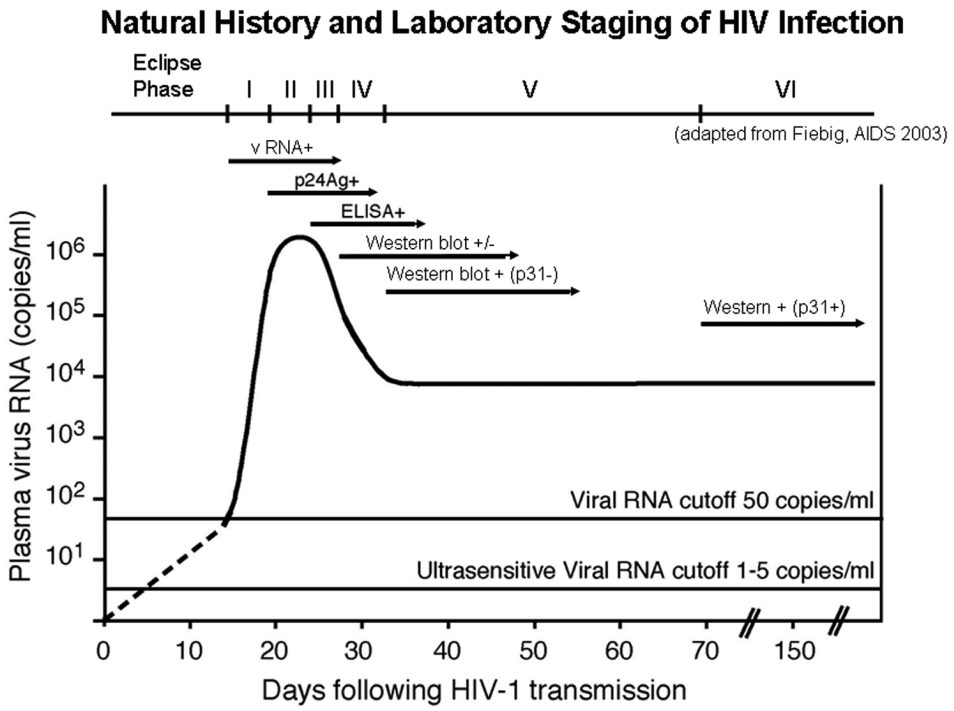

Figure 1. The stages of acute human immunodeficiency virus infection as characterized by detection of viral particles and evolving antibody responses [13]. ELISA, enzyme-linked immunosorbent assay.

Table 1) in which infection is established in local tissue(s) at the exposure site [14], but dissemination at detectable levels in the systemic circulation has not yet occurred. This eclipse phase is thought to last as long as 10 days. Once dissemination to lymphoid tissues and the systemic circulation occurs, HIV replication increases rapidly to a peak level, with a doubling time of 20 h [13]. During the ramp-up phase of viremia, a "window period" exists, during which HIV antibodies are still not yet detectable.

Using stored plasma samples that were obtained twice weekly from unrecognized seroconverting plasma donors, Fiebig et al described 6 stages of acute viremia and early seroconversion characterized by viral replication and evolving antibody responses. In Fiebig stage 1 during ramp-up viremia, only HIV-1 RNA in the blood can be detected (Figure 1, Table 1). About 7 days later, results of tests to detect p24 antigen become positive (Fiebig stage II); p24 antigen is a viral core protein that transiently appears in the blood during the ramp-up phase once HIV-1 RNA levels rise above 10,000 copies/mL [13] and before the development of detectable HIV antibodies.

During the ramp-up phase of AHI, an acutely infected individual develops an intense inflammatory response characterized by high levels of cytokines and chemokines, a "cytokine storm" [15]. In addition, a cell-mediated immune response evoking escape mutants can be demonstrated [16]. Some individuals, but not all, will develop signs and/or symptoms of acute retroviral syndrome, which can include fever, rash, night sweats, severe fatigue, headache, diarrhea, pharyngitis, arthralgia, and myalgias (11). The onset of symptoms of acute retroviral syndrome typically occurs ~2 weeks after HIV acquisition [17], coincident with peak viremia [15].

Within ~5 days after p24 antigen test results become positive, HIV-1 antibodies reach levels that can be detected with sensitive enzyme immunoassays (EIAs) (third-generation EIAs) capable of detecting immunoglobulin (Ig) M antibodies, corresponding to Fiebig stage III (Figure 1). Stage III typically occurs 1–2 weeks after the onset of acute retroviral symptoms. Fiebig stage IV represents the development of an indeterminate Western blot test and occurs ~3 days after sensitive EIA tests show positive results. Conversion to a clearly positive Western blot test, Fiebig stage V, generally occurs after another 7 days, or ~1 month after initial infection (Figure 1 and Table 1).

DEFINITIONS OF AHI

There is no absolute or widely accepted definition of AHI, and this has caused considerable confusion in comparing results. One popular operational definition of AHI is the detection of HIV RNA or p24 antigen in the blood before antibodies have formed (12). It is important to recognize that any definition for the period of acute viremia preceding seroconversion is dependent on the sensitivity of both the HIV-1 RNA or p24

Table 1. Fiebig Stage Classifications for Substages of Human Immunodeficiency Virus Type 1 Primary Infection, with Durations

Stage	Defining finding and/or marker	Duration, mean (range), days	
		Individual phase	Cumulative duration
Eclipse	…	10 (7–21)	10 (7–21)
I	vRNA positive	7 (5–10)	17 (13–28)
II	p24 antigen positive	5 (4–8)	22 (18–34)
III	ELISA positive	3 (2–5)	25 (22–37)
IV	Western blot positive or negative	6 (4–8)	31 (27–43)
V	Western blot positive, p31 antigen negative	70 (40–122)	101 (71–154)
VI	Western blot positive, p31 antigen positive	Open-ended	…

NOTE. ELISA, enzyme-linked immunoassay; vRNA, viral RNA.

antigen assay used to detect viremia and the antibody test employed to detect seroconversion, both of which have markedly improved (see below). The definition is also affected by the variability of early viral replication kinetics and of host immune responses among individuals. A further limitation to the Fiebig staging system is that the data underlying the definitions and durations of each stage are based solely on clade B HIV infections, and HIV RNA, p24 antigen, and antibody assays were developed with clade B viral constituents. Accordingly, it is not certain that this staging system will work as well or that the duration of each stage will be comparable in infections with other HIV clades.

To date, it has been impossible to prospectively study blood or plasma donors with AHI, because these seronegative donors can only be identified as being in the acute stage based on testing performed after their blood donation. Such donors with AHI have inevitably seroconverted by the time they are notified and counseled.

AHI IN COHORTS

A popular strategy to study persons with acute infection is to prospectively follow up cohorts of high-risk subjects for seroconversion, such as men who have sex with men, injection drug users, sex workers, and HIV-discordant couples [18]. The risk of seroconversion can be estimated at the inception of the study based on the prevalence of HIV infection (particularly AHI) in the cohort and the history of incident infections. The limitations of this approach have proved substantial and include (1) greatly reduced risk of HIV acquisition with essential and repetitive safe sex counseling, (2) the cost of following up HIV-negative persons prospectively, and (3) the difficulty in studying subjects at or near the time of seroconversion. Most subjects cannot be seen more frequently than once a month. This can result in subjects being interviewed and samples being obtained at a point far distant from the HIV transmission event and rarely yields critical samples from the seronegative peak-viremia phase of "acute infection." Indeed, subjects are generally enrolled as "acute" because of the detection of antibodies rather than HIV RNA or p24 antigen. To address this problem the US Department of Defense in collaboration with the National Institutes of Health Center for Vaccine Immunology has begun piloting a cohort study (RV217) that uses twice-weekly testing for HIV RNA to detect individuals in the very first days of acute infection (see below).

CROSS-SECTIONAL DETECTION OF AHI

An alternative approach to detecting AHI includes the search for HIV in the blood of individuals at risk or in a general population before HIV antibodies form. This approach de facto identifies people in the earliest Fiebig stages and was developed by blood banks in the late 1990s to eliminate units of blood contaminated with HIV, hepatitis B virus (HBV), or hepatitis C virus (HCV) [19]. Table 2 summarizes the yield of nucleic acid amplification technology (NAT) screening of blood donors through 2008. Blood banks generally perform NAT screening using multiplexed HIV/HCV or HIV/HCV/HBV assays on pools of 6–96 plasma samples derived from individual donations. Reactive pools are resolved to detect individual NAT-reactive donations, which are then retested with virus-specific "discriminatory" NAT assays, leading to donor referral and notification.

This cross-sectional approach for the detection of AHI was first extended to nondonor populations by Quinn et al, who employed a p24 antigen assay to identify subjects with window-phase viremia during the eclipse phase in specimens from an at-risk cohort in India [20]. However, the emergence of p24 antigen occurs later than HIV RNA, and this antigen often disappears as antibodies form, because they complex with the antigen. In 2000, the North Carolina Department of Health and Human Services implemented a state-wide strategy of screening for AHI at all public HIV testing sites [21], the foundation of an ongoing AHI detection program.

Table 2. Summary of the Yield of Nucleic Acid Amplification Technology (NAT) Screening of Blood Donors in Studies Published through 2008

Region and country	No. of positive NAT results (yield rate[a]), by virus		
	HIV	HCV	HBV
Africa			
Republic of South Africa	43 ($1/0.03 \times 10^6$)	1 ($1/0.73 \times 10^6$)	WP: 20 ($1/0.04 \times 10^6$); occult: 36 ($1/0.02 \times 10^6$)
Asia–Pacific			
Australia	2 ($1/4.1 \times 10^6$)	18 ($1/0.45 \times 10^6$)	NA
Japan	19 ($1/2.28 \times 10^6$)	108 ($1/0.4 \times 10^6$)	797 ($1/0.05 \times 10^6$)
Europe			
France	6 ($1/2.52 \times 10^6$)	7 ($1/2.16 \times 10^6$)	WP: 1 ($1/0.099 \times 10^6$); occult: 1 ($1/0.099 \times 10^6$)
Germany	7 ($1/4.54 \times 10^6$)	23 ($1/1.37 \times 10^6$)	WP: 22 ($1/1.43 \times 10^6$); occult: 21 ($1/1.49 \times 10^6$)
Greece	4 ($1/0.22 \times 10^6$)	12 ($1/0.07 \times 10^6$)	89 ($1/0.01 \times 10^6$)
Italy	14 ($1/0.55 \times 10^6$)	27 ($1/0.4 \times 10^6$)	WP: 8 ($2.3/1 \times 10^6$); occult: 189 ($55.5/1 \times 10^6$)
Poland	1 ($1/3.0 \times 10^6$)	74 ($1/0.08 \times 10^6$)	WP: 14 ($4.73/1 \times 10^6$); occult: 53 ($17.9/1 \times 10^6$)
Spain	8 ($1/0.54 \times 10^6$)	15 ($1/0.46 \times 10^6$)	WP: 10 ($1/1.64 \times 10^6$); occult: 39 ($1/0.04 \times 10^6$)
United Kingdom[b] and Eire	2 ($1/12 \times 10^6$)	14 ($1/1.71 \times 10^6$)	NA
North America			
Canada (except Quebec)	1 ($1/6.1 \times 10^6$)	3 ($1/2.43 \times 10^6$)	NA
Canada (Quebec only)	0 ($0/1.75 \times 10^6$)	0 ($0/2.25 \times 10^6$)	NA
United States	51 ($1/1.29 \times 10^6$)	302 ($1/0.22 \times 10^6$)	NA

NOTE. HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; NA, data not available; WP, window period (ie, HIV antibodies not yet detectable).

[a] The number of donated samples required to be tested on average to yield 1 case positive by NAT; this value is in the denominator, and the numerator is either 1 if any case was detected or 0 if no cases were detected.

[b] England, Wales, and Northern Ireland.

In North Carolina all antibody-negative specimens are tested for HIV RNA using pooled NAT screening and a resolution algorithm. Positive NAT pools are broken down until HIV RNA is detected in a single sample and the individual with AHI can be identified. Using this strategy, individuals with acute viremia and their partners can be promptly referred, counseled, treated, and studied prospectively. This approach has subsequently been used by many other investigators [22–30] (Table 3.) The search for AHI can be expected to increase by 1%-10% the number of subjects with established HIV infection who are identified (Table 3.)

In contrast, the increasing use of point-of-care, rapid HIV antibody tests for HIV screening is likely to decrease the number of early HIV infections detected. Recent work has shown some rapid HIV tests to be considerably less sensitive in identifying early infection detectable with standard antibody tests as well as acute infections detectable with NAT assays [30]. Although rapid HIV tests have considerably expanded access to testing in resource-limited settings and have the advantage of same-day results, there is clearly a need to consider performance cost with early and acute HIV infections. This loss of sensitivity for diagnosing early HIV infection with rapid tests is important given the increased risk of onward HIV transmission during these phases and the increasing use of these tests in populations at high risk for acquiring HIV.

TARGETED SCREENING OF SYMPTOMATIC AHI

At least some individuals who acquire HIV develop nonspecific signs and symptoms. Identification of patients with signs and symptoms of early HIV in a cohort or high-risk setting has been used successfully for recruitment of study subjects. Powers et al developed an algorithm to enhance detection of AHI in a sexually transmitted infection clinic in Malawi, where AHI was diagnosed in 21 (1.45%) of 1448 subjects screened for HIV infection [31]. A study that used a model-based score for predictors of AHI allowed the number of subjects screened to detect AHI to be reduced substantially. Predictors for AHI included presentation with a genital ulcer and discordant results of rapid HIV tests. Discordant results from parallel (concomitant) measurement of HIV with rapid tests, as seen in the aforementioned study, probably reflect varying sensitivities and

Table 3. Added Benefit of Routine Screening for Acute Huamn Immunodeficiency Virus Infection (AHI)

Location, reference	Testing population	No. of subjects	Antibody-positive HIV prevalence, %	Increased yield with AHI, %
North Carolina [22]	All public testing	109,250	0.5	3.6
San Francisco, California [23]	STD clinic	3075	3.4	10.5
Los Angeles, California [23]	STD clinic	1712	0.8	7.1
Seattle, Washington [27]	MSM only	3525	2.3	8.6
Atlanta, Georgia [24]	VCT and STD clinic	2202	3	6.1
Johannesburg, South Africa [28]	VCT and STD clinic	1906	35.2	1.8
Lilongwe, Malawi [25]	STD clinic; male	929	46.8	5
Lilongwe, Malawi [26]	STD clinic All	1450	40.5	3.6
Porto Alegre, Brazil [29]	VCT clinic	933	19.1	2.8
Los Angeles, Florida, and New York [30]	STD clinic and MSM clinic	99,111	1.2	2.2

NOTE. AHI, acute human immunodeficiency virus (HIV) infection; MSM, men who have sex with men; STD, sexually transmitted disease; VCT, voluntary counseling and testing.

specificities and provide a strong clue to AHI and impending seroconversion [26].

INCIDENT HIV INFECTION

Reliable estimates of the incidence of HIV infection in a population are critical for epidemiologic characterization, the evaluation of HIV prevention programs, and the design and evaluation of HIV intervention trials. Incidence data can also be used to monitor transmission patterns and better target HIV prevention efforts [32]. Epidemiologic methods separate infections into incident (new) and prevalent (established) categories. Acquisition of HIV generally goes unrecognized, and many years may pass between the time of infection and the actual diagnosis; however, not infrequently the distinction between incident and prevalent infections are lost. This is not a trivial matter, because the difference greatly affects public health policies and resource allocation. Clearly, a new diagnosis of HIV infection or AIDS itself cannot be construed as an "incident infection." In most cases, new diagnoses are made many years after acquisition of HIV and when the CD4 count has fallen to a level that affects health, causing the individual to seek medical attention. As with AHI, there is no absolute definition or time frame limiting incident HIV infection. Clearly, individuals with acute infection have incident infection, as do those who have recently developed an immune response to HIV (seroconverters). Incident infection might be defined as the period from HIV acquisition through seroconversion, the development of a stable viral load (set point), or the first year after HIV acquisition, if this can be identified.

Several methods to estimate the incidence of HIV infection have been attempted, but they have generally been unsatisfactory and poorly adapted to very large populations. The incidence can be derived using "back-calculation" based on the prevalence of HIV infection, with estimation of the period from viral acquisition to AIDS [33]. This indirect approach is logistically challenging and difficult to standardize [34, 35]. Direct measurement of incidence through the prospective follow-up of a cohort of HIV-negative persons who ultimately seroconvert is expensive, unrepresentative, and difficult to sustain even in resource-rich settings [18, 36, 37]. An alternative epidemiologic method used by Rehle et al employs single-year age prevalence data collected through multistage cluster sampling of household surveys to calculate the incidence of HIV infection [38].

It is possible to estimate the incidence of HIV infection through the detection of p24 antigen and/or HIV RNA before seroconversion is confirmed via HIV antibody assays. However, owing to the very short period when acutely infected persons are antigenemic or viremic but seronegative, this method requires very large sample sizes and is generally impractical because of the need to test all seronegative samples for p24 antigen or HIV RNA.

For many infections, the detection of IgM antibodies is considered to suggest recent infection. Although IgM antibodies form in some patients with early HIV infection, they disappear rapidly [39]. As an alternative, Jannsen et al developed an assay to estimate HIV incident infection based on the detection of newly formed IgG antibodies detected against HIV, the serologic testing algorithm for recent HIV seroconversion (STARHS) (Figure 2) [40]. These investigators reasoned that for some defined period of time, serum IgG antibodies from recently infected persons would behave differently in $\geqslant 1$ assay than antibodies from persons with chronic infection. Their approach was to "detune" the standard HIV EIA by increasing the dilution and changing the assay conditions so that patients with lower concentrations of antibody would fall below a cutoff threshold. Because antibodies during acute infection also demonstrate low avidity, which increases as infection duration lengthens, detuned assays can also exploit lower antibody avid-

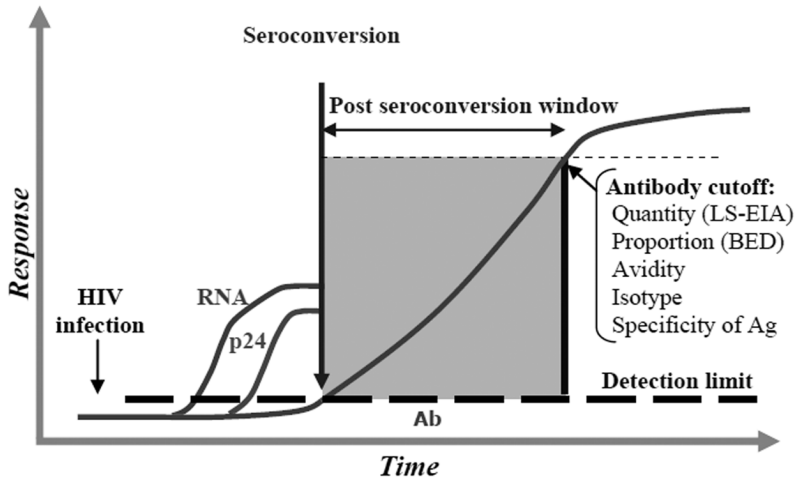

Figure 2. Serologic testing algorithm for recent human immunodeficiency virus (HIV) seroconversion biomarker detection schema. Ab, antibody; Ag, antigen; BED, BED capture enzyme immunoassay; LS-EIA, less-sensitive enzyme immunoassay.

ities to classify individuals as "recently" infected (within the preceding 4 months). Unfortunately, the detuned assay has proved to have poor accuracy. Subsequently, a variety of other assays designed to detect recent antibodies have been developed [33, 41], but each of these has substantial limitations [42], and they do not appear to provide entirely comparable pictures of the incidence of HIV infection [35]. To address these limitations, several groups are developing algorithms using multiple different tests that exclude patients with chronic HIV disease who have low CD4 counts or are receiving treatment, both of which conditions cause falling antibody titers that can be confused with AHI [43].

VIRAL DIVERSITY AND INFECTIVITY IN EARLY HIV INFECTION

Viral isolates can be recovered from patients with AHI, and high-resolution sequencing can be performed on virion-derived HIV RNA in plasma or tissue culture isolates. Studies of viral diversity during acute infection with clade B [12], clade C [44], or clade A [45] HIV have been undertaken using single-genome amplification techniques, allowing greater resolution of the transmitted or acquired virus than heretofore possible. In the majority of cases a single HIV variant is transmitted after heterosexual transmission of HIV, and only rarely are >2–3 variants transmitted. In the first weeks after infection, viral diversification and recombination are inevitable. Accordingly, detection of a single HIV variant generally confirms early infection. Differences between acute or early HIV and chronic HIV can be seen by the significant differences in the "Hamming distance," which calculates the degree of diversity. Obviously, this approach is limited to very special research situations.

IMMUNOLOGY OF THE CLINICAL SYNDROME OF AHI

In serial blood plasma donors with AHI, Stacey and coworkers demonstrated a "cytokine storm" during the earliest days of infection [15]. Furthermore, Gasper et al noted increased levels of markers of apoptosis in peripheral blood manifested by the Fas ligand, tumor necrosis factor receptor–related apoptosis-inducing ligand, tumor necrosis factor receptor 2, and microparticles [46]. C.L.G. and colleagues examined 37 individuals in whom AHI was diagnosed by cross-sectional screening with a relatively narrow window of HIV exposure; the median estimated exposure to HIV occurred 14 days before symptom onset (range, 9–21 days; interquartile range, 12–17 days) (unpublished data). However in these samples from subjects with AHI who were confirmed to have minimal viral diversity, cytokine and Fas ligand levels had returned close to normal, suggesting that the expected elevations in cytokines and viral load were most likely resolved by the time of evaluation.

NEW FOURTH-GENERATION EIAS FOR THE DETECTION OF AHI

Despite evidence that the detection of AHI could limit onward transmission, the ability to find individuals who are acutely infected has been limited by testing methods that are both time and infrastructure intensive. Newer fourth-generation EIAs, combination antigen-antibody tests, detect p24 antigen and anti-HIV-1/2 antibodies concomitantly and, compared with third-generation EIAs, reduce the window period for the detection of early HIV infection by 4 days on average (range, 2 days to 2 weeks) [47–50]. Although fourth-generation assays

will miss fewer HIV infections by detecting p24 antigen (in antibody-negative subjects), a window period will persist during which only HIV RNA can be detected.

Several fourth-generation assays have been approved in Europe and elsewhere. The US Food and Drug Administration approved the Architect HIV Ag/Ab Combo assay (Abbott Laboratories) in June of 2010. A combined analysis from 4 studies demonstrated that this assay had a sensitivity of 99.93% and specificity of 98.79% to detect HIV [51]. In a large, retrospective study, 29 of 57 AHI cases detected via NAT pooling of 97,772 seronegative samples were also tested with the fourth-generation HIV Combo test, of which 25 (86%) of 29 were positive; NAT pooling after negative fourth-generation results increased HIV detection by 0.7%. In sum, the fourth-generation HIV Combo assay has demonstrated excellent sensitivity and specificity for detecting of HIV infection and AHI missed by third-generation assays but misses some AHI cases detected via NAT pooling.

The majority of fourth-generation assays have only positive or negative results and do not discriminate whether the test is positive owing to the detection of HIV antibodies or p24 antigen. Subsequently, in the absence of the symptoms of acute retroviral syndrome and their recognition by clinicians, acutely infected individuals with positive results will not be distinguished from patients with established HIV infection. The fourth-generation Determine HIV 1/2 Ag/Ab Combo assay (Inverness Medical Innovations) provides separate results for the antibody and p24 antigen components. Inverness Medical reports a sensitivity of 100% for detection of chronic infection and specificities of 99.23% for HIV antibodies and 99.66% for HIV-1 p24 antigen [52]. Because the assay incorporates point-of-care technology, it can provide same-day results for established and acute HIV infection. Field testing of this assay is in progress. Although no rapid, point-of-care tests to detect HIV RNA or viral antigens are currently available, in September 2009 the National Institute of Allergy and Infectious Disease of the US National Institutes of Health announced $17 million in funding for such projects for resource-limited settings, where their use could have considerable impact. Several new technologies for rapid, point-of-care assays to detect HIV RNA and p24 antigen are currently being explored [53–57].

CONCLUSION

The detection of acute and incident HIV infections is critical to both prevention and treatment strategies. However, even 30 years after the beginning of the pandemic, our laboratory tools are imperfect, and we are able to identify and care for only a very limited number of persons with recent infection. New diagnostic assays and new surveillance strategies should prove useful, and we anticipate renewed and continued interest in this area of research.

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