

Assays to Estimate HIV
Incidence and Detect
Acute HIV Infection

Global Landscape & Market Assessments



Family Health International

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Acronyms

Ab	Antibody
Ag	Antigen
AIDS	Acquired immune deficiency syndrome
AIS	AIDS Indicator Survey
ARV	Antiretroviral
BMGF	Bill & Melinda Gates Foundation
BSRI	Blood Systems Research Institute
CDC	US Centers for Disease Control and Prevention
CE	Conformité Européenne
CHAVI	Center for HIV/AIDS Vaccine Immunology
DHS	Demographic and Health Surveys
EIA	Enzyme immunoassay
FDA	US Food and Drug Administration
FHI	Family Health International
HIV	Human immunodeficiency virus
HPTN	HIV Prevention Trials Network
HSRC	Health and Social Research Council (South Africa)
IDE	Immunodominant epitope
INGO	International non-governmental organization
LIA	Line immunoassay
LS	Less-sensitive
MICT	Magnetic immuno-chromatography
MSM	Men who have sex with men
NAT	Nucleic acid test; also commonly referred to as “NAAT”
NAAT	Nucleic acid amplification test; also commonly referred to as “NAT”
NGO	Non-governmental organization
NHPICS	National HIV Prevalence, Incidence and Communications Survey (HSRC, South Africa)
NIAID	National Institute of Allergy and Infectious Disease (US NIH)
NIH	US National Institutes of Health
OGAC	US Office of the Global AIDS Coordinator
PEPFAR	US President’s Emergency Plan for AIDS Relief
PMTCT	Prevention of mother-to-child transmission
PrEP	Pre-exposure prophylaxis
RITA	Recent Infection Testing Algorithm
SANBS	South African National Blood Service
STARHS	Serologic Testing Algorithm for Recent HIV Seroconversion
UN	United Nations
UNAIDS	Joint United Nations Programme on HIV/AIDS
UNICEF	United Nations Children’s Fund
WHO	World Health Organization



ASSAYS TO ESTIMATE HIV INCIDENCE AND DETECT ACUTE HIV INFECTION GLOBAL LANDSCAPE & MARKET ASSESSMENTS

Executive Summary

There is a global need for inexpensive, easy-to-use assays for two different, but related purposes: the determination of HIV incidence in populations from surveys, and the identification of persons with acute HIV infection, prior to seroconversion, on standard antibody assays. The pace of development of these two types of assays has differed greatly. To better understand this dynamic, Family Health International (FHI) examined the global landscape and markets for HIV incidence and acute infection assays. Through this endeavor, FHI studied the currently available assays and their uses and attempted to characterize the demand for new and improved assays.

HIV Incidence Assays

Accurate estimates of HIV incidence are essential to fully characterize the epidemic, monitor transmission patterns, prioritize HIV prevention needs, and design and evaluate interventions. However, standard methods for estimating HIV incidence are unsatisfactory. Alternative, assay-based methods were developed a decade ago, but their use has faced technical challenges.

LANDSCAPE ASSESSMENT | In early 2009, FHI conducted 52 qualitative interviews with key informants to assess the landscape of HIV incidence assays. Interviewees represented the breadth of major stakeholders who develop and use HIV incidence assays, including biomedical and behavioral HIV prevention researchers; HIV prevention program leaders; epidemiologists and HIV surveillance experts; basic scientists; donors underwriting major HIV prevention, treatment, and surveillance activities; officials of normative and coordinating bodies; biotechnology and pharmaceutical firms; experts on blood banking; and clinicians.

The interviews revealed that conceptions of acute and recent HIV infection are conflated, and related terms are used inconsistently. The BED assay was the most widely known and the most commonly used incidence assay among interviewees. Although the BED assay was widely known, acceptance and use of this assay were intensely disputed. While some praised its low cost and convenience, current users, former users, and nonusers alike strongly criticized the BED's tendency to overestimate incidence. Interviewees uniformly voiced desire for assays with improved performance, and they were willing to accept incremental increases in cost. About half of interviewees expected that assay performance improvements would lead to increased demand for their use. Donor endorsements and expanded surveillance activities were cited as potential influences on demand.

MARKET ASSESSMENT | FHI consulted with HIV incidence assay users, projected major market segments, and constructed potential scenarios to estimate demand for HIV incidence assays. In contrast to HIV diagnostics that are intended for patient care and management in clinical settings, demand for HIV incidence assays is driven almost exclusively by public health sector and research needs. These needs comprise four major demand components: (1) population-based demographic and health surveys; (2) sentinel surveillance schemes; (3) case-based surveillance; and (4) special studies, including those used to select HIV intervention trial sites, behavioral and biomedical HIV prevention research, and program monitoring and evaluation.

FHI projected the five-year demand by component for three scenarios:

- Scenario 1: The current situation, in which HIV incidence assays are used only on HIV-seropositive specimens and demand is influenced by suboptimal performance.

- Scenario 2: An improved HIV incidence assay is widely available at a low cost and performs consistently well across HIV-1 subtypes. This new assay is used only on HIV-seropositive specimens.
- Scenario 3: A novel HIV incidence assay is developed that can be used both to determine HIV-seropositivity and to identify how recently a given sample was infected. This assay would be used for public health and research purposes instead of individual patient diagnosis.

Projected demand is reported by number of specimens to be tested.

Table 1. Estimated number of specimens to be tested for HIV incidence over five years, by scenario and demand component

Demand Component	Scenario 1		Scenario 2		Scenario 3	
	Lower	Upper	Lower	Upper	Lower	Upper
Population-Based Surveys	8,000	16,000	20,000	50,000	500,000	1,000,000
Sentinel Surveillance	75,000	100,000	250,000	500,000	4,000,000	8,000,000
Case-Based Surveillance	200,000	350,000	300,000	500,000	300,000	500,000
Special Studies	25,000	50,000	150,000	300,000	1,400,000	2,800,000
TOTAL	308,000	516,000	720,000	1,350,000	6,200,000	12,300,000

The limitations of the assays that are currently available have been widely noted and reviewed. Normative bodies have not fully endorsed their use, and consequently, current demand is far below potential future demand. An assay that could be used to identify a sample as HIV-seropositive and from a recent infection could result in an increased demand of 6 to 12 million specimens tested over five years.

Acute HIV Infection Assays

Identifying acute HIV infection by the detection of HIV nucleic acids (e.g., RNA) or antigens (e.g., p24) is important for managing individual patients, implementing public health interventions, and assuring a safe blood supply. Because people with acute HIV infection contribute disproportionately to ongoing HIV transmission, identifying these individuals is a priority for prevention programs. In addition, researchers are interested in better understanding the pathogenesis of acute infection as it relates to HIV transmission and as a potential time to initiate treatment. Moreover, assays that detect HIV nucleic acids and antigens can be used to identify true HIV infection among perinatally exposed infants and HIV vaccine recipients. Most of the acute infection assays that are currently available are expensive and not suitable for use in resource-constrained settings, where the HIV/AIDS burden is greatest.

LANDSCAPE ASSESSMENT | To assess the landscape for acute HIV infection assays, FHI interviewed 20 key stakeholders and reviewed relevant literature, reports, and meeting proceedings. Interviewees included HIV prevention researchers, mother-to-child HIV transmission specialists, experts on blood banking, heads of public health laboratories, HIV epidemiologists, and clinicians who serve high-HIV-risk populations.

Demand in the developed world for early HIV detection for clinical diagnosis and to assure a safe blood-supply has stimulated the development of a variety of sensitive and specific nucleic acid tests and, more recently, combination antigen/antibody “fourth generation” assays. Several fourth generation assays are available in Europe and in other countries, but no such assay has been approved to date for use in the United States. There is a pressing need to develop inexpensive assays that would be suitable for use in Africa and other resource-constrained settings. In September 2009, the U.S. National Institutes of

Health’s (NIH) National Institute of Allergy and Infectious Disease (NIAID) announced \$17 million in funding for projects on rapid, point-of-care HIV virus-detection technologies for resource-limited settings.

MARKET ASSESSMENT | FHI reviewed the data on HIV testing and estimated that there are 132 million HIV diagnostic tests conducted globally each year (Table 2). Furthermore, an estimated 101 million blood and plasma units are tested annually. This results in demand for more than one billion HIV tests over five years.

Acute infection assays are routinely used for blood and plasma testing in the developed world and in some developing countries. Expert opinion varied widely regarding the proportion of HIV diagnostic tests that would be appropriate to test using acute infection assays. However, in view of the very large market for HIV diagnostic tests, if 5 or 20 percent of specimens were tested for acute infection, this would result in a demand for 6 to 26 million tests per year.

Table 2. Estimated number of acute diagnostic tests per year as proportion of total HIV testing, by region

Region	Estimated number of HIV tests per year (2006-7)	Number of acute diagnostic tests per year as proportion of total HIV testing		
		5%	10%	20%
Africa	18,600,000	900,000	1,900,000	3,700,000
Americas	38,300,000	1,900,000	3,800,000	7,700,000
Asia	29,000,000	1,500,000	2,900,000	5,800,000
Europe	41,300,000	2,100,000	4,100,000	8,300,000
Rest of the world	4,900,000	300,000	500,000	1,000,000
TOTAL	132,100,000	6,600,000	13,200,000	26,400,000

Conclusions

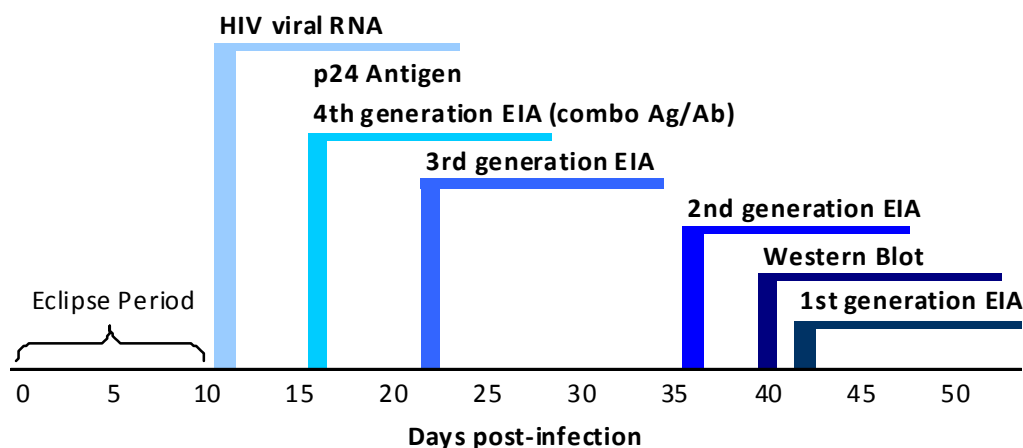
The markets and demand for HIV incidence assays and acute infection assays are profoundly different. HIV incidence assays are used almost exclusively as tools for surveillance, public health, and research, which results in a relatively small demand. Development of HIV incidence assays has taken place largely in government and academic institutions. In contrast, the demand for acute infection assays is driven by a need to protect the blood and plasma supply and to accurately diagnose HIV-infected persons as early as possible. Consequently, demand is very high and has resulted in a very active commercial research and development effort.

As we approach the 30th anniversary of the emergence of the HIV/AIDS epidemic, the need for improved methods to accurately determine HIV incidence is greater than ever. A greater effort is needed to develop and evaluate HIV incidence assays that can more effectively monitor the epidemic and better target prevention and care interventions. For the developing world, where the HIV burden is the greatest, there is a pressing need to make acute infection assays available, affordable, and usable in resource-constrained settings.

Introduction

There is a global need for inexpensive, easy-to-use HIV assays for two different, but related, purposes: (1) reliable identification of specimens that have been collected from recently-infected persons within a defined period after HIV seroconversion to allow for the estimation of HIV incidence in a population from surveys to support the targeting and evaluation of prevention programs (i.e., “HIV incidence assays”); and (2) the identification of persons with acute HIV infection (prior to seroconversion on standard antibody assays) to allow for expedited HIV prevention and care and the interruption of HIV transmission networks (i.e., “acute HIV infection assays”). It is possible that a single test could identify persons before HIV seroconversion and for a defined brief period following seroconversion with important applications both at the individual (clinical management) and public health levels (partner services, characterization of recently transmitted viruses for genotype and drug resistance profiles, and incidence monitoring) Moreover, assays that are capable of identifying HIV nucleic acids (RNA or DNA) or antigens (e.g., p24 antigen) can be used to identify true HIV infection among perinatally exposed infants who are seropositive as a result of maternal antibodies and HIV vaccine recipients who may be seropositive following immunization.

Figure i.1 HIV diagnostics by biomarker appearance (conceptual model)



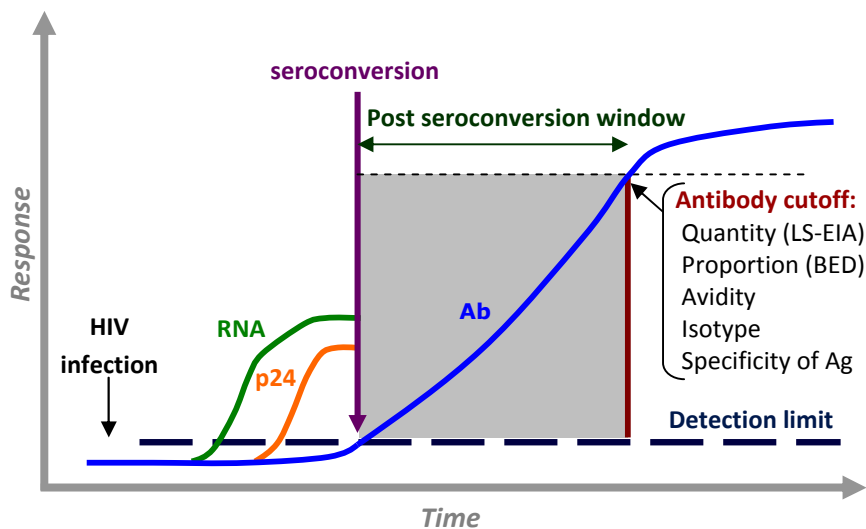
Family Health International (FHI) conducted global landscape and market assessments for both HIV incidence assays and acute infection assays. These assessments were designed to better understand currently available assays and their uses, the needs in the field for new and improved assays, and to estimate the potential market demand for such assays. This information will be helpful in understanding where commercial and public sector investments are currently being made and where there is potential and need for additional investments in assay development and evaluation.

Reliable estimates of HIV incidence in a population are critical for epidemiologic characterization, the evaluation of HIV prevention programs, and in the design and evaluation of HIV intervention trials. Incidence data can also be used to monitor transmission patterns and better target HIV prevention efforts.¹ Recently infected persons contribute substantially to HIV transmission because of behavioral and biologic factors.^{2, 3} Identifying these individuals could have important implications at individual and public health levels.

Standard methods to estimate HIV incidence are unsatisfactory. The indirect approach, which is based on the measurement of prevalence in repeat cross-sectional surveys, is logistically challenging, takes years to conduct, and is difficult to standardize over time. Direct measurement of incidence through the prospective follow-up of a cohort of HIV-negative persons is expensive, unrepresentative, and not sustainable even in resource-rich settings. Furthermore, the enrollment of persons into a cohort study often leads to behavior changes that result in a lower observed rate of HIV incidence than in the broader population of interest. It is possible to estimate HIV incidence from surveys via the detection of p24 antigen or HIV RNA before seroconversion on HIV antibody assays. However, because of the very short period of time in which those persons are antigenemic/viremic before seroconversion, this method requires large sample sizes and is often impractical because of the need to test all seronegative samples for p24 Ag or RNA.

The concept of identifying recently infected persons from among those found to be seropositive on standard serologic assays in cross-sectional surveys was developed a decade ago as a means of estimating HIV incidence in a population.⁴ This method employed the Serologic Testing Algorithm for Recent HIV Seroconversion (STARHS). A number of serologic assays were developed and used in STARHS schema, but this methodology and these assays were promptly shown to have substantial limitations, including biological, epidemiological, and statistical confounders.⁵ Despite their suboptimal performance and ongoing controversy, these assays continue to be used in a variety of settings for diverse purposes.

Figure i.2 STARHS biomarker detection schema⁶



The identification of acute HIV infection is a priority for numerous reasons. First, people with acute HIV infection contribute disproportionately to ongoing HIV transmission because of their greater infectiousness related to a higher HIV viral load.^{7, 8} Having recently acquired HIV infection themselves, they might be engaging in risky behavior, and are therefore likely to be sources of transmission to those within their social networks who are HIV-uninfected. Better identification of people recently infected is therefore a priority area for HIV prevention programs.⁹ Second, acute HIV infection might also be an optimal time to initiate HIV care services.¹⁰ Third, there is currently intense interest in studying the pathogenesis of acute HIV infection to better understand the virus and host factors that are associated

with HIV transmission in order to develop improved biomedical prevention measures, including HIV vaccines, microbicides, and pre-exposure prophylaxis (PrEP) with antiretroviral drugs.¹¹ Ongoing research is yielding a better understanding of the early immune response to HIV infection, the characteristics of transmitted viruses, and virus-host interactions associated with viral dissemination and initial control of viremia.¹² Finally, identification of acute infection is essential to protecting the blood supply.¹³ Diagnostics used to identify HIV infection among infants who have been exposed perinatally to HIV and HIV vaccine recipients draw on technologies similar to those used for acute HIV infection assays and contribute to global demand.

There has been considerable interest in better understanding acute HIV infection and in characterizing the maturation of the immune response to HIV infection as a basis for assays to distinguish specimens from persons with recent infection from those with long-standing infection. However, there has been insufficient attention given to the overlap of these two closely-related areas and to using the best available scientific findings to develop improved assays. The field of assay development could be greatly advanced by stimulating new, creative thinking, assessing the global demand for such assays, and setting a framework for assay development, calibration, evaluation, and validation.

The purpose of this report is to contribute to a better understanding of the perceptions of, need for, and potential demand for HIV incidence and acute infection assays. It is anticipated that this improved understanding will stimulate targeted research and development that will lead to improved assays and enhanced HIV prevention, care, and treatment for the world's populations.

ASSAYS TO ESTIMATE HIV INCIDENCE

Chapter 1: Landscape Assessment

Tracking HIV incidence is critical to understanding epidemiological trends and assessing the effectiveness of interventions. Traditional epidemiologic methods for estimating HIV incidence have proved unsatisfactory. As an alternative, researchers have developed laboratory assays that are based on the principle that the HIV antibody response matures over time and that people with recently acquired infection can be identified during a defined post-seroconversion “window period.” These tests could then be used to determine the number of recently infected persons among the HIV-seropositives identified in a representative sample of a population or from a surveillance system with the resulting number and window period duration used to estimate HIV incidence.

A decade ago, scientists at the US Centers for Disease Control and Prevention (CDC) and collaborators reported on the use of such an assay in a method known as the Serologic Testing Algorithm for Recent HIV Seroconversion (STARHS).⁴ This algorithm employed a sensitive commercial assay (Abbott HIV 3A11) and a customized, less-sensitive (LS or “detuned”) version of this assay. The specimen from a recently infected individual is reactive on the sensitive assay, but non-reactive on the LS assay. A similar approach was used with the bioMérieux Vironostika HIV-1 assay.^{14, 15} Researchers subsequently found that these assays (both based on HIV-1 subtype B, the most common form in the United States and Europe) had significantly different post-seroconversion window periods for non-B subtypes, which predominate in other regions of the world.¹⁶⁻¹⁸ Consequently, without accounting for subtype distributions among the HIV-seropositive subjects in the sample, use of these assays would yield inaccurate estimates of HIV incidence. An additional practical problem was that both of the commercial assays that formed the basis for the algorithm were removed from the market by their original manufacturers.

Other immune-response-maturation approaches to identifying recent HIV-1 infection have been based on:

- The proportion of HIV-specific antibodies that appear against parts of gp41 from HIV-1 subtypes B, E, and D among all IgG as measured by a capture enzyme immunoassay (EIA) (the “BED” assay);¹⁹
- Relative quantification of the avidity of anti-HIV antibodies using a modified third generation anti-HIV assay;²⁰⁻²²
- Measurement of antibody response to a gp41 immunodominant epitope (IDE) and various gp120-V3 loop peptides, known as IDE-V3 assay;²³⁻²⁵
- Measurement of isotype IgG3 anti-HIV, which is present early in the immune response;²⁶ and
- Quantification of anti-HIV antibodies on a line immunoassay (Inno-LIA HIV adaptation).²⁷

These STARHS approaches have been challenged by the following obstacles:

- Variability of the immune response among HIV-1 infected individuals and the impact of antiretroviral therapy and late stage AIDS immunosuppression leading to a lack of sensitivity and specificity (especially long-term specificity) in identifying persons with recent infection;
- Variation in the window period for different HIV-1 subtypes or populations;

- Difficulty in the standardization of post-seroconversion window-periods, assay calibration, and quality control measures;
- The unpredictable availability of the commercial products that are used as the backbone for some HIV incidence assays;
- The complexity and high cost of some of the assays; and
- The perceived lack of a commercial market for HIV incidence assays.²⁸

These factors have made the use of HIV incidence assays the subject of some debate and controversy.

Guidance is evolving on the use of the only commercialized HIV incidence assay, the BED assay. The Joint United Nations Programme on HIV/AIDS (UNAIDS) reference group's 2005 statement on the use of the BED assay for HIV incidence estimation recommended against using the BED assay for routine HIV surveillance applications.²⁹ This statement was made after a review of BED-based HIV incidence estimates indicated that such estimates were substantially higher than those obtained with other methods. In 2006, a statement from the Office of the US Global AIDS Coordinator (OGAC) suggested that the BED assay could be used with appropriate adjustments in conjunction with expert consultation in sentinel or population-based surveillance and evaluation of HIV prevention interventions.³⁰ More recent guidance has recommended caution when using the BED assay in considering the populations and uses by determining use of antiretroviral therapy, determining CD4 counts, employing expert consultation on sample sizes and using adjustments for misclassification. The World Health Organization (WHO) Technical Working Group on HIV Incidence Assays, in conjunction with CDC scientists, developed a methodologic guidance document for standardizing the validation of existing and future HIV incidence assays.³¹ There is an ongoing WHO-led effort to develop guidance on when and how to use assays for recent infection to estimate HIV incidence at a population level.

Table 1.1 HIV incidence assays

Assay	Type of antibody measured	Commercially available	Special equipment required*
Calypte Aware™ BED™ EIA and SEDIA™ BED HIV-1 Incidence EIA**	Anti-HIV gp41 quantity (as a proportion of total IgG)	Yes	No
Abbott 3A11 HIV Ab (detuned)	Anti-HIV quantity	No [†] ; previously available as modified commercial product	Yes
Avioq HIV-1 Microelisa System (detuned; formerly Vironostika EIA)	Anti-HIV quantity	Yes, although not consistently [†]	No
Abbott AxSYM HIV 1/2/g0 EIA Avidity	Antibody avidity (anti-HIV quality)	Yes; modified commercial product	Yes
Ortho Vitros EIA Avidity	Antibody avidity (anti-HIV quality)	Yes; modified commercial product	Yes
IDE-V3	Anti-HIV gp41 and V3 quality	No	No
Anti p24 IgG3	Anti-HIV p24 IgG3	No	No
Inno-LIA	Presence or absence and	Yes; modified	No

	quantity of antibody	commercial product	
Particle agglutination assay	Quantity	Yes; modified commercial product	No
Uni-Gold Recombigen	Quantity	Yes; modified commercial product	No

*Special equipment excludes ELISA plate readers and washers.

**During the course of the Landscape Assessment for Assays to Estimate HIV Incidence reports circulated that Calypte had ceased producing the BED HIV incidence assay. As of December 2009 Calypte had repackaged and begun marketing this product as the Aware™ BED™ EIA.

†In 2009 the Vironostika EIA was relicensed by Avioq and repackaged as the Avioq HIV-1 Microelisa System.³²

In assessing the global landscape for assays that are used to estimate HIV incidence in populations, FHI conducted a series of qualitative interviews with key stakeholders. The methods that were used to design and conduct these interviews are described below. Findings are presented below interviewee opinions of available HIV incidence assays and their relative strengths and weaknesses; the breadth of applications for which they are used; desired and necessary improvements to these assays; barriers to development of new assays; and predicted demand for currently available versus improved HIV incidence assays.

Methods

FHI began its global landscape assessment for HIV incidence assays by seeking input from a select group of expert advisors, which included representatives from the Bill & Melinda Gates Foundation, Blood Systems Research Institute (BSRI), CDC, WHO, and the market research firm *bioStrategies* Group. Working with this team of expert advisors, FHI determined that a formal data collection activity was required to assess the breadth of opinions and circumstances affecting demand for HIV incidence assays.

In-depth interviews with 52 key informants were conducted in March and April of 2009. Each interview lasted approximately one hour. Interviewees were identified to ensure input from major stakeholder groups, including biomedical and behavioral HIV prevention researchers; HIV prevention program leaders; epidemiologists and HIV surveillance experts; basic scientists; large donors underwriting HIV prevention, treatment, and surveillance activities; multilateral coordinating bodies; biotechnology and pharmaceutical firms; and clinicians. A breadth of industries, organizations, and geographies were represented by the diverse individuals interviewed (see Table 1.2).

Geographic regions represented by interviewees
<ul style="list-style-type: none"> ▪ Americas ▪ Europe ▪ North Asia ▪ Southeast Asia ▪ Sub-Saharan Africa ▪ Oceania

Table 1.2 HIV incidence assay interviewees: examples by organization type

Organization types	Examples
Multilateral bodies	WHO The Joint United Nations Programme on HIV/AIDS (UNAIDS)
Donors	US Agency for International Development (USAID) Bill & Melinda Gates Foundation
International NGOs	Macro International

	Population Services International (PSI)
Government agencies	Ministries of Health US CDC US National Institutes of Health (NIH)
Academia	University of Kwazulu-Natal (South Africa) Imperial College of London (UK)
Blood safety	South African National Blood Service (SANBS) Blood Systems Research Institute (BSRI)

FHI and *bioStrategies* Group developed, piloted, and refined a structured interview guide. The instrument used open-ended questions to address the following issues:

1. Terminology around HIV incidence assays and recent HIV infection;
2. Awareness and use of specific assays to estimate HIV incidence;
3. Purposes for which HIV incidence assays can, are, and should be used;
4. Advantages and disadvantages of specific HIV incidence assays;
5. Quantity of samples tested and assays used in estimating HIV incidence;
6. Relative importance of various assay attributes, including cost, training requirements, specificity, sensitivity, performance across subtypes and more;
7. Incremental versus substantial improvements required in refined or newly developed HIV incidence assays;
8. Predicted changes in the need and demand for HIV incidence assays; and
9. Factors influencing predicted changes in the need and demand for HIV incidence assays.

Findings

Direct quotes from interviewees are highlighted throughout *in text boxes*.

TERMINOLOGY

Interviews began with discussions of the terms that are used to describe recent infection, and relevant detection assays. Conceptions of acute and recent infection were conflated across interviews, as were the terms around their detection. The term “recent” was used and defined in many different ways. For many interviewees, the recent status of an HIV infection was based on the performance of a specific assay. Consequently, the “recent period” varied according to the assay that was used. Some defined an infection as recent according to time post-infection. Of these, the most commonly cited timeframe for a recent infection was six to 12 months post-transmission. A smaller number of interviewees considered infections one to two months post-transmission as recent. Interviewees acknowledged and were concerned about this variability in labeling. One interviewee reported avoiding the term “recent infection” completely because of the confusion in terminology.

I don't use the term [recent infection]. I don't like it. It's too vague.
- HIV PREVENTION RESEARCHER, US

Interviewees were asked what they would call an assay that is used to estimate HIV incidence in a population. Many, but not all, agreed that an assay that is specifically used to estimate HIV incidence should be referred to as an “incidence assay.” Others recommended using the terms “recent infection assay,” “early infection assay,” “acute infection assay,” “early seroconversion test,” and “multi-assay

algorithm.” One expert urged that assays that are used to estimate HIV incidence should be named such that users clearly understand their purpose.

APPLICATIONS & SPONSORS

Interviewees reported using HIV incidence assays for a variety of public health and research purposes. The most frequently cited application was for epidemiological surveillance. Many interviewees saw significant value in using HIV incidence assays to monitor general epidemiological trends and to better understand dynamics among high risk populations. Other uses that they cited were for assessing the impacts of prevention measures in program monitoring, identifying appropriate sites for clinical and behavioral interventions, estimating HIV transfusion risk, and validating and developing assays. Organizations funding activities that involve HIV incidence assays include national public health and international development agencies, foundations, and major multilateral organizations.

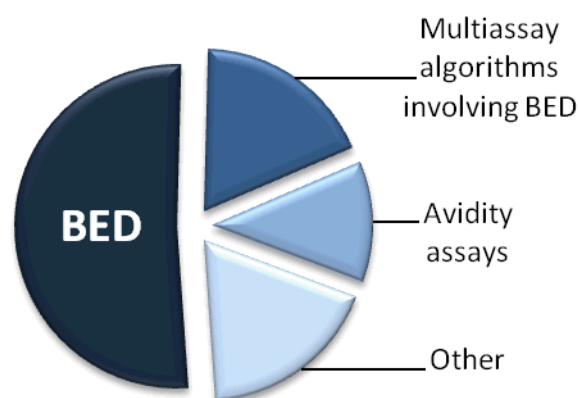
Examples of sponsors funding activities involving HIV incidence assays

- Normative bodies (UNAIDS, WHO)
- Multilateral agencies (The Global Fund to Fight AIDS, Tuberculosis, and Malaria)
- Foundations (Bill & Melinda Gates Foundation)
- National health and development agencies (USAID, CDC, and NIH; UK Department for International Development and Health Protection Agency; Canadian International Development Agency; French Ministry of Health and Solidarity)
- Blood banking organizations (BSRI, Etablissement Français du Sang, SANBS)

AWARENESS & USE

When asked to name specific HIV incidence assays of which they were aware, interviewees uniformly noted the BED assay as one. Other assays tended to be noted according to an individual’s area of expertise, e.g., basic scientists mentioned detuned assays most commonly, and HIV surveillance prevention experts seemed very familiar with avidity assays. Other methods noted were RNA tests, PCR, rapid tests, and p24 antigen-based assays. Of those interviewees using HIV incidence assays directly in their own work, approximately one-half reported using the BED assay alone to estimate HIV incidence. Others used it in multi-assay algorithms. Avidity and detuned assays were also used, but less commonly.

Figure 1.1 Proportion of HIV incidence assays used by interviewees



All interviewees had heard of the BED assay, but not all of them were aware of alternative methods to estimate HIV incidence, such as avidity tests and less-sensitive (“detuned”) assays. After the BED assay, interviewees most commonly used multi-assay algorithms involving the BED and avidity tests. Fewer interviewees used avidity tests alone or detuned assays to estimate HIV incidence, while some reported using other methods to estimate HIV incidence, some components of which are not commercially available.

ILLUSTRATIVE QUOTES

The BED [is] pretty cheap and it's really quite simple.

- EPIDEMIOLOGIST, EUROPE

The BED is...really grabbing people's interest because they really would love to be able to measure [incidence].

- EPIDEMIOLOGIST, EUROPE & ASIA

I don't think [BED assays] give you a valid estimation of incidence rates. Generally, they overestimate... I've lost confidence because of that.

- HIV PREVENTION PROGRAM LEADER, AFRICA

[Overestimation] makes it harder to go to the national authorities and say 'This would be a great thing to implement on a wide scale.' You can't do that until you have data on how the BED actually performs in your setting.

- EPIDEMIOLOGIST, EUROPE & ASIA

[Requiring use of multiple correction factors] defeats the whole purpose of having an assay that is transferable from region to region and subtype to subtype.

- LABORATORY SCIENTIST, US

So many people have used [the BED assay] and abused it and got bad results that they're saying "This bloody thing doesn't work!"

- HIV PREVENTION PROGRAM LEADER, AFRICA

BED

The primary advantages of the BED assay that the interviewees cited were convenience and cost. They commented positively on its high throughput and ease of use in terms of training and sample types that were required. Low estimated cost per sample and commercial availability were also appreciated. Others highlighted the fact that, despite its shortcomings, the BED assay can provide insight into temporal trends or intra-group comparisons in the same population.

Conversely, current users, former users, and nonusers alike strongly criticized the BED assay's tendency towards overestimation of HIV incidence. Interviewees were particularly concerned about the significant number of samples that were incorrectly identified as recent infections ("false recent"), especially among ART users and elite controllers. These concerns were widely and strongly expressed and prompted some interviewees to refuse to use the BED assay.

In addition to concerns regarding overestimation of incidence, interviewees expressed other misgivings regarding BED. They cited correction factors that were required in using BED as being both inadequate and too complex. Interviewees noted difficulty in inter- and intra-user reproducibility of results from the BED assay. Others noted the BED assay's inconsistent performance across HIV subtypes.

Concerns also arose around the use of the BED for purposes other than estimating HIV incidence in populations. This could be attributed to interest in identifying individuals with recent HIV infection and the lack of available methods to do so. One interviewee complained that the BED was not suitable for field use because it was not available in a rapid test format. Others maintained that the BED is not sensitive or specific enough to be used as a diagnostic tool. One interviewee felt that negative views of the BED assay are in part a result of it being used for inappropriate purposes.

Interviewees raised positive and negative comments related to the BED assay's commercial availability. Following development at CDC, the BED was first marketed by Calypte Biomedical Corporation. Some interviewees specifically cited liking the BED assay because of its commercial production. However, by the date of the interviews in early 2009, rumors had begun circulating that Calypte had or would

soon cease operation. Several interviewees were concerned about potential supply interruptions if this happened. In mid-2009, Sedia Biosciences began commercially producing BED kits. As of November 2009, three other companies had licensed the assay, but they had yet to market them.³³

Supply interruption concerns are not specific to BED alone. Abbott and bioMèrieux halted production of the 3A11 and Vironostika assays, respectively, detuned versions of which were used to estimate HIV. In 2009, the biotechnology company Avioq, Inc. successfully acquired a new Food and Drug Administration (FDA) license to commercialize a version of the Vironostika assay.³²

The breadth of challenges involved in using the BED assay significantly impacted interviewees' opinions. Known and perceived limitations spurred some interviewees to avoid relying on assay-derived cross-sectional HIV incidence data entirely.

MULTI-ASSAY ALGORITHMS

A diverse set of interviewees, including epidemiologists and laboratory scientists working in academia, NGOs, and multilateral bodies operating in the US, Europe, and Asia, reported using or being very interested in multi-assay algorithms. BED in combination with an avidity assay was cited as the most commonly used algorithm. Other components of a multi-test algorithm under development and evaluation are CD4+ cell count determination to eliminate persons with advanced HIV disease and HIV RNA testing to eliminate persons with well-controlled viremia. Others suggested the determination of antiretroviral (ARV) use, by questionnaire or serum assays, to eliminate persons on therapy. The use of these non-serologic markers would be part of a Recent Infection Testing Algorithm (RITA) that builds upon and moves beyond the STARHS concept. Among users and interested parties, the main cited advantage of multi-assay algorithms was its improved accuracy over the BED alone. The main reported drawback of using multi-assay algorithms was the lack of substantial experience with and validation of these algorithms.

We're moving away from the idea that any single assay will be able to [measure incidence].

- US LABORATORY SCIENTIST

AVIDITY ASSAYS

Interviewees also reported using avidity tests alone. Users of this assay remarked on its availability, effectiveness, and reliability. It was perceived as convenient because it is a modification of a commercially available product, and as effective and reliable because it is more sensitive than the BED and less susceptible to overestimation. Reported limitations of the avidity assay included limited user experience and concern with its complexity. Some interviewees thought that correct use of avidity assays would require more training than the BED assay. Other concerns were how to establish a standardized cutoff and the harshness of reagents that are necessary for processing.

Maybe it would be better on a long-term basis to be independent of a company, to have the same assay for a long period of time, and this is why we chose to develop our own assay... for availability and cost-effectiveness.

- LABORATORY LEADER,
EUROPE

If you say, "I'm giving you a test which will tell you for sure that this person is a recent infection" you could put the cost up by a factor of ten and I would still buy it.

- HIV PREVENTION
PROGRAM LEADER, AFRICA

OTHER ASSAYS

In addition to the BED assay, multi-assay algorithms and avidity assays, interviewees discussed several other assays used to estimate HIV incidence. These assays include detuned versions of commercial HIV antibody assays, as well as so called "home made" assays. The latter are produced using widely available laboratory products. Interviewees noted that the Abbott 3A11 and bioMérieux Vironostika detuned antibody assays were no longer commercially available. Some commented that these detuned assays were inferior to other available options such as the BED. Interviewees involved in the in-house development of home-made assays explained that their doing so was prompted by supply disruptions and cost concerns. These home-made assays were developed in Europe and used strictly for population-level surveillance of HIV incidence.

DESIRED ATTRIBUTES

Interviewees indicated that sensitivity, specificity, and reproducibility of results supersede cost as priorities for improved HIV incidence assays. When asked to describe desired attributes in HIV incidence assays, epidemiologists, researchers, and laboratory scientists uniformly voiced desire for assays with specificity and sensitivity superior to those of BED. Potential users reported willingness to accept incremental increases in cost for: (1) higher specificity, (2) greater sensitivity, and (3) improved reliability of results over those found in currently available assays. Other important assay attributes that users described were ease of sample type required, consistent availability, easily interpretable results, and high throughput. Interviewees also desired clear guidance on which assays to use for specific applications and populations.

The approximate costs of incidence assays range widely and are influenced by multiple factors. Because the BED assay is the only designated incidence assay that is currently available on the commercial market, cost comparisons should be interpreted with caution. The BED and other EIA-based immunoassays require use of standard EIA plate readers and washers and highly trained technicians. The reported estimated cost for BED and other EIAs ranges from \$7 to \$20 per sample. The Inno-LIA is reportedly the most expensive of those EIAs that are used to estimate incidence, costing an estimated \$40 per sample. For assays that require proprietary equipment, such as the Abbott AxSYM and Ortho Vitros EIAs, the required equipment could cost more than \$100,000 if purchased independently. However, these assays are primarily used for HIV diagnostic testing. Their producers are known to waive the cost of required equipment in lieu of large contracts to purchase reagents and other materials. Particle agglutination assays are reportedly the least expensive method to estimate incidence in terms of cost per sample. They can be performed using standard laboratory equipment and widely available reagents for as little as \$1 to \$2 per sample.

DEMAND

Interviewees were evenly split about whether demand for currently available incidence assays is likely to change in the future. However, given an improved incidence assay, the majority thought demand would increase. Interviewees who predicted increased demand indicated that better assays could improve understanding of epidemiological trends, allow for more targeted programming to high-incidence groups, and enhance the cost-effectiveness of HIV prevention research and programs.

A small number of interviewees remained skeptical that global demand for assays to estimate HIV incidence would increase, even with improved assays. This was mostly based on the perception that a new assay would simply replace currently used assays. One interviewee noted that demand would be reduced in areas where the epidemic is lessening, and approximately one-third of interviewees could not make a prediction on demand for these assays.

Interviewees had a variety of opinions about factors that would impact demand for an improved HIV incidence assay. These factors include endorsement of specific assays by normative bodies, such as WHO, UNAIDS, and by major donors, such as the Global Fund to Fight AIDS, Tuberculosis and Malaria, and the US President's Emergency Plan for AIDS Relief (PEPFAR). Other demand drivers they cited included expanded surveillance activities including both sentinel surveillance and more large-scale population-level, cross-sectional surveys. Some saw improved, accurate, and affordable HIV incidence assays as a possible factor leading to expanded surveillance efforts because surveillance would provide more valuable information.

Others predicted that evidence for the importance of early treatment and prevention could have a major impact on demand, especially if a newly developed assay could be used to identify recent infection in individuals. This potential game-changing scenario is discussed further in Chapter 2.

If [an improved] test came out, I think the funding would flow quickly because this is such a huge limitation to the programs and to the research...it's rare that you have one thing that is holding you back as much as the limitations of these tests do.

– HIV PREVENTION PROGRAM LEADER, US

The market is there for these [incidence] tests. Certainly from the public health side...the Global Fund would be ecstatic if there was a reliable test.

– INTERNATIONAL EPIDEMIOLOGIST

Demand [for incidence assays] hasn't changed appreciably in the last ten years. The thirst for an assay has been fairly constant.

– INTERNATIONAL EPIDEMIOLOGIST

A real hit may not be so much for surveillance purposes, but for contact tracing...There may be a difference in treatment requirements for recently infected people...You're talking about a whole different application segment.

– INTERNATIONAL EPIDEMIOLOGIST

ASSAYS TO ESTIMATE HIV INCIDENCE

Chapter 2: Market Assessment

FHI consulted with HIV incidence assay users, projected major market segments, and constructed potential scenarios to estimate demand for HIV incidence assays. In contrast with HIV diagnostics intended for patient care and management, demand for HIV incidence assays is driven almost exclusively by public health sector needs. HIV incidence determination based on assays falls into four major areas:

- Population-based demographic and health surveys;
- Sentinel HIV surveillance schemes;
- Case-based HIV surveillance; and
- Special studies, including those used to select HIV intervention trial sites, behavioral and biomedical HIV prevention research, and program monitoring and evaluation.

These four areas comprise four components of demand for HIV incidence assays.

Decisions on the use of HIV incidence assays in programs, surveillance systems, and research are made primarily by funding bodies and sponsors. This is in contrast to HIV diagnostics where individual health care facilities and providers may decide on the diagnostic assay to use. However, in developing-country settings, there, too, funders and sponsors frequently chose HIV diagnostic tests. For HIV surveillance systems, national public health agencies, usually in conjunction with normative bodies and sponsors, determine which incidence estimation methods are used. In other instances, individual investigators and laboratory leaders might make such decisions independently. Endorsements from normative bodies, such as WHO and UNAIDS, are likely to influence assay selection across applications.

Methods

FHI leveraged expertise from current and potential future user groups to estimate global demand for HIV incidence assays. Expert informants included the following:

- HIV surveillance experts from national and international public health agencies with case-based and sentinel surveillance systems;
- Individuals responsible for designing and conducting population-based surveys and biomedical and behavioral HIV prevention research and programs at international NGOs and academic institutions;
- Heads of HIV epidemiology, research, and surveillance laboratories; and
- Principle Investigators leading HIV prevention research, including vaccine trials.

A minority of these key informants also participated in qualitative interviews discussed in Chapters 1 and 3. Further information sources included peer-reviewed journal articles and published reports on HIV surveillance practices, laboratory and field-based experiences with HIV incidence estimation methods, comparative performance assessments of available assays, and proceedings of relevant working groups and normative bodies. Substantial input was received during the May 13-14, 2009, Meeting of Experts on Development of Assays to Estimate HIV Incidence, convened by FHI and partners in Chapel Hill, North Carolina (meeting proceedings available at http://www.fhi.org/en/HIVAIDS/pub/meeting_reports/HIV_inc_assays.htm).

For each demand component in which HIV incidence assays are likely to be used the total number of samples for HIV testing was estimated over a five-year period. Within these totals, the number of HIV - positive specimens was also estimated. These figures were then used to project global demand given three different scenarios discussed in this chapter's findings.

For **population-based surveys** the total number of biological specimens collected for HIV testing was tabulated across major surveys conducted from 2003 to 2008 (see Table 2.1).

Table 2.1 Population-based surveys used to estimate demand for HIV incidence assays

Major population-based surveys	<ul style="list-style-type: none"> ▪ Demographic & Health Surveys (DHS) ▪ AIDS Indicator Surveys (AIS) ▪ Human Sciences Research Council (HSRC) National HIV Prevalence, Incidence and Communication Survey (NHPICS)
Number of countries surveyed	35
Years surveyed	2003-2008
Mean survey sample size	15,318
Total survey sample size	536,117
Total HIV prevalence	4.0%

The number of HIV positive specimens in each survey was calculated by multiplying the HIV prevalence found in the survey by the total number of biological specimens collected. The five-year frequency of surveys was estimated for each country and applied to the expected number of specimens collected for HIV testing and the number of HIV-positive samples. For example, the HSRC NHPICS for South Africa is conducted every three years. In the most recent survey (2008) 15,031 samples were tested for HIV, 10.9% of which were found positive. Thus the five-year estimate for the number of HIV+ samples is $15,031 \times 10.9\% \times (5/3) = 2,731$. Additional illustrative examples are displayed in Table 2.2

Table 2.2 Estimated number of HIV samples collected over five years: sample calculations

Country	Most recent survey data			Survey frequency (every X years)	5-year estimates	
	# of samples collected for HIV testing	Sample HIV prevalence	# of HIV+ samples		# of samples collected for HIV testing	# of HIV+ samples
India	National Family Health Survey (NFHS)-3 (2005-6)			5	102,000	286
	102,000	0.3%	286			
South Africa	HSRC NHPICS (2008)			3	25,052	2,731
	15,031	10.9%	1,638			
Tanzania	AIS (2007)			4	13,434	940
	10,747	7.0%	752			
Uganda	AIS (2004-5)			5	18,525	1,186
	18,525	6.4%	1,186			

For **case-based** and **sentinel surveillance**, it was assumed that only HIV-positive specimens would be shared with a central laboratory for incidence testing. Generally, such schemes collect specimens on a periodic or ongoing basis. Countries that were employing or considering establishing HIV incidence testing were determined through literature reviews, insight from national and international HIV surveillance experts, and communication with assay developers and producers. These sources also guided estimates for the number of HIV-positive specimens tested annually in national case-based surveillance systems and sentinel surveys.

For **special studies** that would possibly employ HIV incidence testing, the sample size and HIV prevalence for current and likely future major HIV studies and special projects were estimated over a five-year period based on a recent review of HIV incidence testing and interviews with a wide range of HIV researchers, public health officials, and representative of normative organizations.

Findings

POPULATION-BASED SURVEYS

Population-based HIV surveys collect information and blood specimens from a representative sample of the population of interest. Data are collected on a wide array of indicators including demographic characteristics and health indicators. Standardized survey methods allow for comparison of results across countries, within specific subpopulations or sub-regions, and over time. Surveys yield important information regarding the magnitude of HIV epidemics and their trends. In order to yield adequately reliable estimates at national and sub-national levels, population-based surveys employ large sample sizes (typically 5,000 to 20,000 persons). These surveys are more appropriate for monitoring HIV in countries with generalized epidemics versus those with epidemics concentrated among subpopulations or in select areas; however, some countries with concentrated epidemics have conducted population-based surveys.

Because of their scale, expense, and the difficulty of collecting data in remote resource-limited regions, population-based health surveys in countries with generalized HIV epidemics are most commonly conducted every three to five years. Some information regarding HIV incidence can be gleaned by examining a country's HIV prevalence data from multiple time points. Estimates of HIV incidence from a cross-sectional survey, alternatively, indicate more timely epidemiological information on incidence, e.g., where and among what subpopulations HIV was transmitted most frequently over the previous six to 12 months. This type of information is extremely valuable in identifying HIV transmission trends and "hot spots" where incidence rates are high or have increased. Populations with low incidence are also noteworthy for identifying successful strategies for controlling HIV transmission. Such information can be valuable for the design and evaluation of HIV prevention interventions and programs.

Only a small proportion of population-based surveys that collected samples for HIV testing recently used HIV incidence assays; all used the BED assay. These surveys included the Kenya AIDS Indicator Survey (KAIS),³⁴ Uganda AIS,³⁵ and South Africa's NHPICS.³⁶ The KAIS included the collection of an additional tube of blood to allow for CD4+ cell count determination among those found to be HIV-seropositive. This information allowed for further refinement of the HIV incidence estimate. Since 2001, more than

half a million HIV samples have been collected through Demographic and Health Surveys (DHS), AIDS Indicator Surveys (AIS), and others. For STARHS-based incidence assays, HIV prevalence and survey size determine the potential demand for incidence assays (see Table 2.3). Population-based surveys create relatively limited demand for currently available HIV incidence assays (used only on HIV-seropositive specimens) despite the large number of specimens collected in the surveys.

Table 2.3 Number of HIV-seropositive samples in select population-based surveys

Survey	Number of samples HIV tested	Sample HIV prevalence	Number of HIV+ Samples
Ghana DHS (2003)	9,566	2.2%	210
Kenya AIS (2007)	18,000	7.4%	1,332
Swaziland DHS (2006-7)	9,177	25.9%	2,377

SENTINEL SURVEILLANCE

Developed and developing countries use sentinel surveys to track the HIV epidemic. Sentinel surveillance is used in countries with both generalized and concentrated HIV epidemics. Commonly surveyed sentinel populations include general and high risk groups and those from whom biological samples are regularly collected. These include women attending antenatal clinics, STI clinic clients, female sex workers, male truck drivers, military personnel, police, prisoners, and men who have sex with men (MSM). The most recent WHO/UNAIDS guidelines on HIV sentinel surveillance recommend collection of both biological specimens and behavioral data, referred to as integrated biologic and behavioral surveillance (IBBS).^{37, 38}

As funding for HIV prevention and treatment programs in resource-poor countries has increased, opportunities for sentinel surveillance have also grown. Between 2003 and 2006, the number of antenatal clinics serving as sentinel sites for 30+ African countries more than doubled, from 1,000 to 2,500.³⁹ Despite the large number of biological specimens collected, relatively few sentinel surveillance programs are known to apply HIV incidence assays. A notable exception is China, which tests samples collected from sentinel sites across all 31 provinces.⁴⁰ An avidity assay has been used to estimate incidence among Portuguese STI clinic attendees.⁴¹ The BED assay has been used to estimate incidence for samples collected from antenatal clinic attendees in Botswana, Côte d’Ivoire, Ethiopia, South Africa, Zambia, Zimbabwe, Cambodia, Thailand, and Brazil.^{33, 42-44} This incidence testing is done mostly on an *ad hoc* basis and is not an integral component of the sentinel surveillance systems.

CASE-BASED SURVEILLANCE

HIV surveillance based on the reporting of newly identified HIV infections is implemented in many developed countries. Inadequate public health and reporting infrastructure in most developing countries precludes the use of case-based surveillance. Modern case-based surveillance for HIV infection grew out of AIDS case reports early in the epidemic. As highly active antiretroviral therapy (HAART) became widely available in the developed world and delayed the onset of clinical AIDS, newly diagnosed cases of HIV infection became a more accurate marker for epidemiological trends, especially in countries

with concentrated epidemics. Accurate case-based reporting of individuals who are newly diagnosed with HIV requires a highly robust public health infrastructure. Practitioners must be able to diagnose HIV cases reliably using standardized methodologies applied consistently across an entire nation, from urban to rural and wealthy to impoverished regions. A national reporting schema must be easily accessible and assiduously maintained by a central public health agency to avoid counting an HIV infected person multiple times.

Despite widely accessible HIV testing, most HIV-positive individuals are unaware of their infection status, making new HIV case reports a poor proxy for population incidence.²⁸ In the United States and Canada, more than half of all new HIV diagnoses are reported within one year of an individual developing clinical AIDS, indicating long-standing infections.⁴⁵ HIV incidence can be estimated from HIV case reports and the testing of collected biological specimens with an HIV incidence assay. In current schemas, this involves assessing samples with one of the HIV incidence assays discussed in Chapter 1. Regardless of the assay applied, complex weighting and correction factors are required to extrapolate population incidence from samples collected.

In the United States, case-based surveillance of new HIV diagnoses is carried out by CDC. In addition to tracking reports of newly diagnosed HIV infections, CDC collects left-over blood specimens from a sample of new cases in more than 20 areas that account for the majority of new HIV cases nationwide.⁴⁶ These newly identified HIV-positive samples are then tested with the BED-EIA to estimate HIV incidence in the population. Similar case-based HIV surveillance schemas are employed in Canada and some European countries.^{16, 47} The HIV incidence assays used in these programs vary widely and include some custom-developed tests that are not commercially available. In France, a virologic surveillance system has been in place since 2003. Dried serum spots from HIV-positive specimens are directed to the French National Reference Center and tested with a proprietary assay capable of identifying recent infections (<180 days post-transmission). As a result, French public health authorities have identified demographic characteristics associated with higher transmission rates.²⁴ Several middle-income and developing nations are also reportedly exploring the use of case-based surveillance to estimate HIV incidence, including China, Russia, Brazil, Nigeria, and some Caribbean nations.

SPECIAL STUDIES

HIV incidence assays are used in biomedical and behavioral research to identify high-risk populations and to assess outcomes. HIV prevention trials require populations with substantial HIV incidence to evaluate interventions. Only those sites and populations with incidence above a certain level will allow meaningful measurement of an intervention's effectiveness in a timely and efficient manner. Available assays have been used to assess sites for HIV vaccine trial appropriateness with mixed results.⁴⁸ HIV incidence assays can also be used to estimate incidence as an outcome of prevention trials. An example is Project Accept (HPTN-043), a US NIH-funded randomized trial of community mobilization and HIV counseling and testing in Africa and Thailand. Project Accept will rely on HIV incidence estimation in communities from cross-sectional surveys of approximately 50,000 persons as the main outcome of interest. HIV incidence assays have also been used to identify recently infected persons for inclusion in treatment trials, and to monitor and evaluate treatment and prevention programs.

DEMAND SCENARIOS

Demand for HIV incidence assays was estimated using three scenarios. These scenarios assume a five-year horizon and address the number of specimens that will be tested with HIV incidence assays, rather than the number of tests that will be used. The number of tests required to evaluate these specimens would need to be adjusted to account for required duplicate or triplicate testing with the same assay, quality controls runs, training, multiple-test algorithms, inefficient use of kits (fewer tests run than the kit can accommodate), and wastage (e.g., expired kits).

Scenario 1 represents the current situation in which HIV incidence assays are used only on HIV-seropositive specimens. Demand for tests represents ongoing and expected future uses, assuming that the current barriers to use continue.

Scenario 2 represents the situation if an improved HIV incidence assay was available that had improved sensitivity and specificity over existing tests and methods, and consistently high performance across all HIV-1 subtypes. The new test (or tests) would be easy to use, available at a low cost, and would be endorsed by normative bodies for use for epidemiologic and surveillance purposes. Projected demand in this scenario assumes widespread availability of the assay.

Scenario 3 represents a “game changing” situation. It projects demand for an HIV incidence assay that can be used to determine *both* HIV-seropositivity *and* recent status. This assay would be used for public health and research purposes rather than individual patient diagnosis. Scenario 3 assumes that the assay in question would be (a) highly sensitive and specific in determining seropositivity and recent status across diverse populations; (b) easy to use, requiring limited technical training, equipment and materials to use; (c) widely available at low cost; and (d) endorsed by normative bodies for epidemiologic and surveillance purposes.

Lower and upper estimated demand is shown for each scenario (see Tables 2.4-2.7). In scenario 1, projected five-year demand is estimated to range between 308,000 and 516,000 HIV-seropositive specimens to be tested. The number of assays that are actually required to test these specimens would be approximately two- to four-fold higher because of the factors discussed above. The largest proportion of specimens to be tested, about two-thirds, would come from case-based surveillance systems. Sentinel surveillance systems account for one-fifth to one-quarter of specimens. Population-based surveys and special studies, including research studies, account for a small proportion of specimens.

Table 2.4 Estimated number of specimens to be tested in Scenario 1

Scenario 1	Estimated Five-Year Demand	
	Lower	Upper
Population-Based Surveys	8,000	16,000
Sentinel Surveillance	75,000	100,000
Case-Based Surveillance	200,000	350,000
Special Studies	25,000	50,000
TOTAL	308,000	516,000

In scenario 2, estimated demand increased more than two-fold to between 720,000 and 1,350,000 specimens. There was a substantial increase in specimens from case-based surveillance, but an even greater increase from sentinel surveillance as it is estimated that with an improved assay many more countries would initiate incidence testing as a part of sentinel surveillance.

Table 2.5 Estimated number of specimens to be tested in Scenario 2

Scenario 2	Estimated Five-Year Demand	
	Lower	Upper
Population-Based Surveys	20,000	50,000
Sentinel Surveillance	250,000	500,000
Case-Based Surveillance	300,000	500,000
Special Studies	150,000	300,000
TOTAL	720,000	1,350,000

In scenario 3, with a “game changing” assay used for HIV diagnosis plus incidence determination, estimated demand increased by an order of magnitude of between 6.2 million and 12.3 million.

Table 2.6 Estimated number of specimens to be tested in Scenario 3

Scenario 3	Estimated Five-Year Demand	
	Lower	Upper
Population-Based Surveys	500,000	1,000,000
Sentinel Surveillance	4,000,000	8,000,000
Case-Based Surveillance	300,000	500,000
Special Studies	1,400,000	2,800,000
TOTAL	6,200,000	12,300,000

INDIVIDUAL DIAGNOSIS

If an assay had sufficient sensitivity and specificity to be used for individual diagnosis, it could be used routinely in clinical settings. Such an assay could be used as an additional test following the diagnosis of HIV-seropositivity on standard tests; or, as in scenario 3, such a test could be both an HIV diagnostic and a test for recent infection. Use of these tests in clinical settings, apart from epidemiologic and surveillance applications, opens the possibility for far wider testing uses. Opinions of the experts we interviewed varied widely on the likely use of assays for recent infection for individual patient characterization. Chapters 3 and 4 discuss the vastly larger potential clinical testing arena for individual diagnosis. The market for a clinical use assay for recent infection would likely greatly eclipse the estimated market described here in terms of five-year demand and could be in the range of millions of tests *per year*.

Table 2.7. Estimated number of specimens to be tested over five years, by scenario and demand component

Demand Component	Scenario 1		Scenario 2		Scenario 3	
	Lower	Upper	Lower	Upper	Lower	Upper
Population-Based Surveys	8,000	16,000	20,000	50,000	500,000	1,000,000
Sentinel Surveillance	75,000	100,000	250,000	500,000	4,000,000	8,000,000
Case-Based Surveillance	200,000	350,000	300,000	500,000	300,000	500,000
Special Studies	25,000	50,000	150,000	300,000	1,400,000	2,800,000
TOTAL	308,000	516,000	720,000	1,350,000	6,200,000	12,300,000

Current tests for recent HIV infection that are used to determine HIV incidence are applied only to HIV-seropositive specimens, thereby making the epidemiologic and surveillance applications as efficient as possible. However, a relatively low demand for the current generation of HIV incidence assays results from this efficiency. Furthermore, the widely noted and publicized limitations of current incidence assays (especially the BED) resulted in a lack of endorsement of their routine use by normative bodies (i.e., UNAIDS), which has further limited demand. We estimate that an improved incidence assay would result in at least a doubling of demand. An assay that could be used for the diagnosis of both HIV-seropositivity and recent infection (for epidemiologic purposes) could increase demand by an order of magnitude to six to 12 million specimens tested over five years.

Moving to an assay that would be endorsed for use for individual clinical diagnosis opens the potential market dramatically. As discussed in Chapters 3 and 4, more than 100 million HIV diagnostic tests (apart from blood and plasma screening) are performed *per year* globally.

ASSAYS TO DETECT ACUTE HIV INFECTION

Chapter 3: Landscape Assessment

FHI conducted a landscape assessment on the uses of and demand for assays to detect acute HIV infection. These include tests capable of identifying HIV nucleic acids (primarily RNA) and antigens (primarily p24).

Methods

Information was collected through interviews with key stakeholders and reviews of relevant literature, reports, and meeting proceedings. Target interviewees were identified by soliciting recommendations from widely recognized experts in HIV medicine, epidemiology and acute HIV infection assays, with additional recommendations provided by interviewees themselves. Twenty interviews were conducted between April and September 2009. Each interview lasted for approximately one hour. Participants included HIV prevention researchers; PMTCT program experts; international leaders in the blood banking industry; heads of public health laboratories; HIV epidemiologists; and clinicians serving high HIV risk populations. The majority of interviewees were interested in the use of acute HIV assays globally. Some were focused on their use specifically in low and middle-income countries; in US public health settings; and on commercial opportunities in developed markets. Additional interviewee characteristics are summarized in Table 3.1.

Table 3.1 Characteristics of key stakeholders interviewed regarding acute HIV assays

Industries	Academia Biotechnology and pharmaceutical companies International non-governmental organizations (INGOs) National and state health agencies	
Fields of Expertise	Biomedical engineering Clinical medicine Epidemiology Immunology	Infectious disease Marketing Microbiology Public health
Sample Institutions	Abbott Diagnostics National Institutes of Health (NIH) Elizabeth Glaser Pediatric AIDS Foundation Stanford University	
Sample Roles	HIV clinician, academic medical center Global product manager, biotechnology corporation Medical director, state public health HIV/STD branch Program officer, HIV immunology research consortium	

An informal interview guide included questions on terminology related to acute HIV diagnostics and epidemiology; specific assays used to detect acute HIV infection; applications for which such assays are and can be used; advantages and disadvantages of various assays; necessary and desired attributes in improved assays; unmet need for acute HIV infection assays; potential increases or decreases in future

demand for such assays; and any extant factors influencing the development and availability of assays to detect acute HIV infection.

Findings

DEFINING ACUTE HIV INFECTION

As described in Chapter 1, the terms “HIV incidence assay” and “acute HIV infection assay” are used and interpreted inconsistently across audiences. For the purposes of this report, the term “acute HIV infection” refers to the period following HIV transmission and before HIV antibodies (Ab) are detectable by commercially available assays. Using this definition, the duration of acute HIV infection is determined by the sensitivity of the antibody assay used. Acute infection would be substantially shorter using more-sensitive third generation antibody assays than using older second or first generation assays. Feibig, et al., defined primary HIV infection stages based on the detection of viral RNA, p24 antigen (Ag), and HIV antibodies via enzyme EIAs (see Figure 3.1 and Table 3.2).⁴⁹ However, these, too, are susceptible to variable interpretation based on assay performance characteristics.

Figure 3.1 Fiebig stages of primary HIV infection^{12, 49}

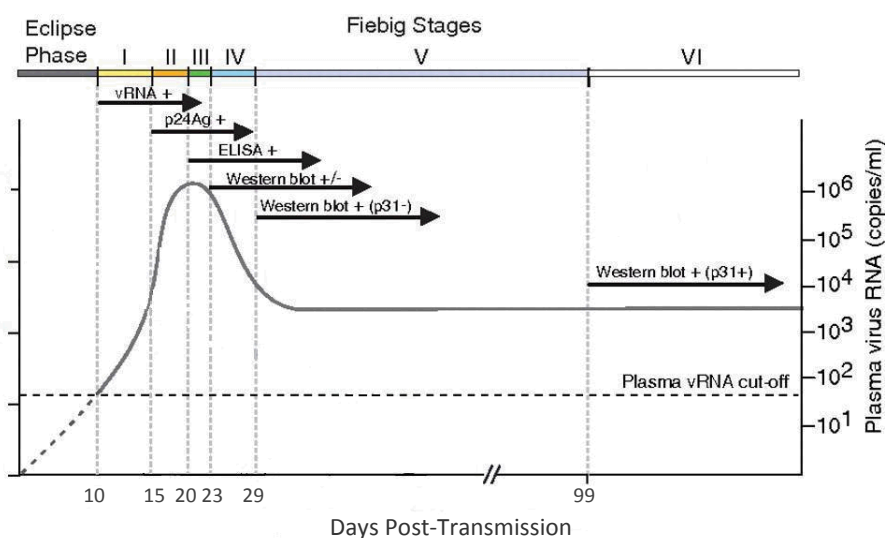


Table 3.2 Fiebig stages of primary HIV infection⁴⁹

STAGE	Approximate duration in days (cumulative)	BIOMARKER DETECTION				
		RNA	p24 Ag	HIV Ab (generation EIA)		Western blot
				3 rd	2 nd	
Eclipse	10 (10)	-	-	-	-	-
I	5 (15)	+	-	-	-	-
II	5 (20)	+	+	-	-	-
III	3 (23)	+	+	+	-	-
IV	6 (29)	+	+/-	+	-	Indeterminate
V	89 (99)	+	+/-	+	+/-	+ (without p31 Ag band)
VI	Ongoing	+	+/-	+	+	+

Some, but not all, sources define the acute period of HIV infection in terms of days or weeks post-transmission. The exact time period in question varied substantially across interviewees and published reports, from four days to six weeks post-transmission. Several interviewees commented on the practice of linking terminology with the purposes for which assays are employed. That is, terming assays specifically used to estimate incidence in populations “HIV incidence assays,” versus calling assays used in clinical settings “acute infection assays.” Some disliked the notion of differentiating these assays, as well as differentiating acute versus recent infections.

METHODS TO DETECT ACUTE HIV INFECTION

Traditional serologic diagnosis of HIV infection relies on initial screening with an enzyme immuno-assay (EIA) for HIV antibodies, followed by confirmatory “reflex testing” for positive or indeterminate samples. Currently, acute HIV infections are identified by detection of HIV RNA or p24 antigen in samples that appear negative on HIV antibody assays. Viral RNA is detected with nucleic acid [amplification] tests (NAT or NAAT). A variety of p24 antigen assays have been available for many years. Fourth generation EIAs, termed “combo Ag/Ab” tests, are capable of identifying acute HIV infection through p24 antigen detection. Many fourth generation assays identify a sample as HIV positive based on detection of *either* HIV antibodies or p24 antigen, and do not report results separately for Ab and Ag. Thus, these assays do not discriminate between acute HIV and longer-standing infection. Supplemental HIV antibody, RNA, and/or p24 antigen testing is required to make this distinction. An acute HIV sample would present as negative on a standard HIV antibody assay, but positive on a fourth generation EIA or NAT. Examples of assays capable of identifying acute HIV infections detection appear in Table 3.2.

I try to simplify the terminology and use one common terminology, so you don't have these concepts [acute infection and HIV incidence] being siloed.

- INGO EXECUTIVE

It's difficult to understand what the value [is]...to distinguish people between the first and second week [post-transmission] from people further along... It's...100% completely arbitrary and clinically probably irrelevant...

- US HIV CLINICIAN & PREVENTION RESEARCHER

Table 3.3 Examples of methods and assays to detect acute HIV infection⁵⁰

Method	Manufacturer	Name	Regulatory approval for HIV diagnosis	
			CE (Europe)	FDA (US)
4 th Gen. Combo Assay (Ag/Ab)	Abbott	ARCHITECT HIV Combo	✓	✗
		AxSYM HIV Ag/Ab Combo	✓	✗
		PRISM HIV Ag/Ab Combo	✓	✗
	bioMérieux	VIDAS HIV DUO ULTRA & Quick	✓	✗
		VIDAS HIV DUO	✓	✗
		VIDIA HIV DUO	✓	✗
		Vironostika HIV Uniform II Ag/Ab	✓	✗
	Bio Rad	Genscreen Ag/Ab HIV Ultra	✓	✗

		Genscreen Plus HIV Ag/Ab	✓	X
	Roche	Elecsys 2010 HIV Combi	✓	X
		Cobas Core HIV Combi	✓	X
	Siemens	Enzygnost HIV Integral	✓	X
		Enzymun Test HIV Combi	✓	X
	Adaltis Italia	HIV Combo 4 th Generation	✓	X
	Inverness	Determine HIV-1/2 Ag/Ab Combo	✓	X
	Murex	Murex HIV Ag/Ab Combination	✓	X
	Organics	Immunocomb II Trispot	NA*	X
	Green Cross	Genedia HIV Ag-Ab ELISA	NA	X
HIV RNA	Gen-Probe	Aptima HIV-1 RNA Qualitative Assay	NA	✓
		Procleix	NA	X
	Roche	Ultra Sensitive Amplicor HIV-1 Monitor	NA	X
		COBAS Ampliscreen	NA	X
	bioMérieux	NucliSens HIV-1 QT	NA	X
	Siemens	Versant HIV-1 RNA bDNA Assay	NA	X
p24 Ag	bioMérieux	VIDAS HIV p24 Assay	NA	X
	Perkin Elmer	Alliance HIV-1 p24 Ag ELISA Kit	NA	X
	Advanced BioSciences	Antigen Capture Assay, HIV-1 p24	NA	X

*NA = Definitive information unavailable.

Additional novel assays under development are discussed below.

APPLICATIONS

The potential uses for assays that detect acute HIV infection span clinical, public health, and research applications.

Clinical diagnosis | For clinicians in both developed and developing world settings, early diagnosis of HIV is crucial in managing individual patients and preventing onward HIV transmission. Persons with acute HIV infection are more infectious as a result of high viral levels and they account for a substantial proportion of HIV transmission. The estimated proportion of transmission resulting from acute infections varies in different settings according to the stage of the epidemic and exposure network factors. There is ongoing debate on the relative importance of acute infection testing vs. expanded serologic testing to identify more HIV-infected persons. Some experts in the United States argue that the acute infection testing is not cost effective in the vast majority of clinical settings and they favor expanded testing programs using sensitive serologic assays. Others have found that the traditional serological testing algorithm that the United States uses can be improved to detect HIV cases earlier in the course of infection and at a lower cost.⁵¹

Pooled NAAT screening has been used to identify acute infections among persons found to be HIV-seronegative in a variety of HIV testing venues. These include clinics targeting at-risk populations in Atlanta, Los Angeles, Seattle, and Washington DC.⁵² Since 2002 the state of North Carolina has used

pooled NAAT screening to identify acute infections for those seeking HIV testing at more than 100 publicly funded sites.⁹ Among STD clinic patients in Malawi, 40.6% were HIV seropositive and 1.5% had acute infection identified by use of pooled NAAT testing.⁵³

For medical practitioners working with patients at high risk for HIV infection, rapid point-of-care acute HIV infection assays would serve as a useful tool to evaluate persons with suspected acute retroviral syndrome. Currently, many diagnostic opportunities are missed because of the lack of such assays and the inadequate awareness of clinicians. Some experts felt that new rapid acute infection assays would serve to raise the awareness of clinicians to diagnose acute infection. Early diagnosis of acute HIV infection would allow for HIV prevention counseling aimed at interrupting HIV transmission. The Center for HIV/AIDS Vaccine Immunology (CHAVI) has identified a large number of acutely infected persons in research settings, mainly through the use of pooled RNA testing. A CHAVI study (CHAVI-011) has explored behavioral aspects of acute HIV infection. This study has led to the development of the HIV Prevention Trials Network (HPTN) study 062, which will evaluate optimal counseling interventions for acutely infected persons.⁵⁴

While consensus has not yet been reached, some research suggests that early initiation of anti-retroviral therapy has the potential to improve health outcomes for acutely infected individuals.^{10, 55} A recently approved US NIH-funded study in Malawi will explore initiation of ART for patients identified to be acutely infected.⁵⁶

The monitoring of plasma HIV viral RNA levels (viral load) is a key component of clinical management and monitoring of HIV infected persons in developed countries. As discussed below, novel viral detection assays are being developed to monitor treatment outcomes in resource-limited settings where current RNA testing is prohibitively expensive.

Public Health | Acute infection diagnosis can play an important role in the prevention of mother-to-child transmission (PMTCT). For many women in developing countries, antenatal clinics are the primary site for HIV diagnosis and access to healthcare. In countries with high HIV prevalence and incidence, unidentified acute HIV infections in pregnant women contribute to a substantial portion of perinatal infections.⁵⁷ Simple, rapid, point-of-care acute infection assays would be widely used in developing country ANCs. There are several ongoing clinical trials of HIV pre-exposure prophylaxis (PrEP) using daily, oral ARVs among HIV-uninfected persons. For persons on ARV PrEP who become HIV infected, it will be important to stop PrEP as soon as possible following infection to minimize the selection of drug resistant viruses. Consequently, rapid, point-of-care acute infection assays would be of great value.

Early Infant Diagnosis | For infants born to HIV-infected women, early and accurate HIV diagnosis is essential to assuring prompt care and treatment. The presence of maternal anti-HIV antibodies requires the use of viral nucleic acid or antigen detection through the first 15 to 18 months of life. The large burden of HIV infection borne by women in the developing world, especially Africa, creates great demand for simple, easy-to-use nucleic acid or antigen detection assays.

HIV Vaccine Trials | HIV vaccine recipients may be positive on serologic assays as a result of vaccine-induced antibody production. Assays that can quickly and rapidly identify true HIV infection by RNA or antigen detection among vaccine trial participants would be very helpful for prompt diagnosis and efficient trial conduct. Rapid point-of-care assays would be particularly useful for this application.

Research | Research on acute HIV infection continues to be a high priority for better understanding the early immune response to HIV infection, the characteristics of transmitted viruses and virus-host interactions associated with viral dissemination, and initial control of viremia.¹² Identification of acutely infected individuals could lead to improved biomedical prevention measures, such as HIV vaccines, microbicides, and pre-exposure prophylaxis (PrEP) with antiretroviral drugs.^{11,49}

Blood and Plasma Screening | Perhaps the most important application of acute HIV detection is to assure a blood and plasma supply that is safe from HIV transmission. The demand for a safe blood supply requires using the most sensitive methods available. Current pooled NAAT methods are capable of detecting all infections except those that occur during the eclipse period. Such testing is used widely in developed countries. A number of developing and middle-income countries are exploring means to improve their blood and plasma donation and screening systems. These include implementing pooled NAAT screening for HIV in South Africa and Namibia.^{33, 58} However, more affordable assays are greatly needed for both standard diagnosis and acute HIV detection, with the potential to substantially improve blood safety.

FUNDING AND DEVELOPMENT

Demand for early HIV detection in the developed world has stimulated a great deal of research and development activity from major diagnostics companies as well as small biotechnology firms. In particular, demand in the European market has resulted in registered products from Abbott, Roche, Siemens, BioRad, Inverness, bioMérieux, and GenProbe. Manufacturers are not solely based in or focused on Western markets; the South Korean-based Green Cross Sang-A Corporation produces the fourth generation Genedia assay. These tools, however, are targeted primarily for use in advanced clinical settings and diagnostic laboratories. They do not include rapid, point-of-care assays appropriate for developing country settings where the burden of high-level HIV transmission, mother-to-child-transmission, and the need for early infant diagnosis is greatest.

In 2007, the US NIH's National Institute of Allergy and Infectious Disease (NIAID) convened a meeting on "Novel Technologies in Rapid HIV-1 Viral Detection" to review the current state-of-the-art and to identify unmet needs. In 2008, NIAID issued a request for applications (RFA) on "Rapid HIV Point-of-Care Diagnostic Device for Resource-Limited Settings." This funding opportunity was intended to bridge existing technologies rather than fund the development of entirely new platforms, recognizing that the fundamental technical capabilities already existed to quickly and cheaply detect acute infection. In September 2009, NIAID announced three awards for advanced development of rapid point-of-care HIV virus detection assays. Awardees included Diagnostics for the Real World (DRW), which received \$4.7M

to explore a point-of-care nucleic acid amplification dipstick with preloaded assays that produces visual results within two hours. Wave80 Biosciences received \$7.5M to further develop a signal amplification assay for viral detection that is run in a cartridge and small, portable reader. This assay is intended for use in early infant diagnosis or monitoring increases in viral levels for persons on ART in resource-limited settings. Advanced Liquid Logic received \$5.2M to adapt its digital microfluidics “lab-on-a-chip” for HIV viral detection in low-technology settings. Other technologies appropriate for use in resource-poor settings are under development with support from foundations and other government agencies. Scientists at Northwestern University received a Gates Grand Challenge grant to further develop viral and p24 antigen assays. The latter requires nominal battery-powered hardware and is reportedly able to yield results comparable to those produced by Abbott’s fourth generation assays in less than twenty minutes. Scientists at the CDC are developing a rapid point-of-care p24 assay that uses magnetic immuno-chromatography (MICT).⁵⁹

DEMAND

Substantial market demand has driven the development of multiple fourth generation combo Ag/Ab and HIV RNA assays that are capable of detecting acute HIV infection. In the developed world, individuals and health care systems are willing to pay a premium price for early and accurate detection of HIV.⁶⁰ Major diagnostic manufacturers are interested in making fourth generation assays available in the United States in coming years. Key stakeholders noted ample US market opportunities, including hospitals, public health departments, blood donation centers, and commercial reference labs, as well as the nation’s approximately 600 sexually-transmitted disease clinics. Experts remarked that demand will increase as standards of care change, with more aggressive screening for acute HIV infection among emergency rooms patients, pregnant women, and other high risk populations.

Additional evidence of the benefit of early treatment and transmission intervention might further increase demand. Showing that ARV PrEP is safe and effective in preventing HIV infection and implementing it programmatically would add to the demand for assays to detect acute HIV infection; it will be important to diagnose HIV infection as soon as possible for person on PrEP. Performance standards set by, and endorsements from, normative bodies will have significant influence on assays of choice.

BARRIERS TO DEVELOPMENT AND ACCESS

The cost and time commitment of premarket approval and post-market surveillance is a substantial barrier to developing assays and making them available for detecting acute infection in the United States. Experts report that the regulatory scheme that is used in the US for HIV diagnostics has placed the US far behind Europe in getting new products to market. As one expert noted “We were using a [an HIV antibody] test that the rest of the world including the developing world dropped more than 10 years ago.” Several experts advocated for the reduction in regulatory hurdles for HIV diagnostics in order to expedite moving new products to market.

Other barriers to development and production of new acute infection assays include validation and operational barriers. Seroconversion panels are an expensive and scarce commodity, but they are essential to the development and complete validation of new assays. Operational barriers include the time and cost that are required to establish systems for notifying and reporting diagnoses of acute infection; tracing and contacting partners; and setting up laboratories with the capacity to conduct the tests in volume. One interviewee suggested a highly interactive meeting to determine what to do next in the realm of assays to detect acute HIV infection; this could be modeled on the May 2009 Meeting of Experts on the “Development of Assays to Estimate HIV Incidence” held in Chapel Hill, North Carolina. Such a meeting would concentrate primarily on the developing world and address how to get diagnostics into the hands of those who need them most.

Assays that detect acute HIV infection have a breadth of potential applications that span clinical diagnosis, public health, blood safety, and research. The large size of these market opportunities is reflected by the number of diagnostics that are currently available and under development. Governments and foundations have funded the development of acute assays for resource-limited settings, especially to aid in early infant diagnosis.

ASSAYS TO DETECT ACUTE HIV INFECTION

Chapter 4: Market Assessment

Methods

In order to estimate potential market demand for assays to diagnose acute HIV infections, Family Health International (FHI) and bioStrategies Group first estimated the total number of HIV tests that have been conducted globally. This estimate was based on the number of persons tested for HIV infection for diagnostic purposes in addition to the number of blood and plasma units collected for donation and processing.

To estimate the number of HIV tests that are performed per year, we included for analysis the top 100 countries by number of persons living with HIV infection as estimated by UNAIDS; this included 35 countries in sub-Saharan Africa and 65 outside of sub-Saharan Africa. Data were assembled on the total populations and the number of persons between the ages of 15 and 64. For sub-Saharan African countries, the percent tested in the past 12 months was sourced from the Demographic and Health Survey database. If this number was not available for individual countries, then the median for the country's African region (e.g., western, eastern, southern, or central) was applied. Outside of sub-Saharan Africa, the percent tested for the last 12 months was obtained from multiple sources.⁶¹⁻⁶³ When testing data were not available for a particular country, the percent tested was derived from a regression equation based on two factors: (1) the percent of the population HIV infected and (2) the Human Development Index (HDI) rating. The HDI is an index that is used to rank countries by level of "human development," which usually indicates whether a country is developed, developing, or underdeveloped.⁶⁴

The estimated number of annual blood donations and plasma collections were obtained from the World Health Organization's (WHO) Global Database on Blood Safety and Blood Safety Indicators.⁶⁵ This source was also used to estimate the proportion of blood donations occurring in the developed and developing world, as well as the proportion of units tested for HIV antibodies and for acute infection by antigen and/or nucleic acids.

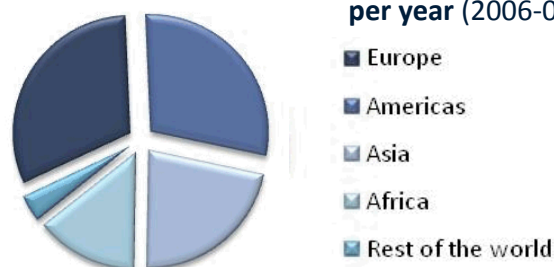
Findings

As of 2006-07, there were an estimated 132 million persons tested for HIV infection per year, including approximately 19 million in Africa, 38 million in the Americas, 42 million in Europe, 29 million in Asia, and 5 million in the rest of the world (see Table 4.1 and Figure 4.1). Countries with large numbers of estimated annual HIV testing include the United States, 21 million; Russia, 18 million; China, 16 million, Brazil, 8 million; India, 5 million, Nigeria, 3.8 million; and South Africa, 2.5 million.

Table 4.1 Estimated global HIV diagnostic tests per year (2006-07)

Africa	18.6 M
Americas	38.3 M
Asia	29.0 M
Europe	41.3 M
Rest of the world	4.9 M
TOTAL	132.1 M

Figure 4.1 Estimated global HIV diagnostic tests per year (2006-07)



However, there has been a sharp increase in HIV testing programs in recent years, particularly in Africa as part of a global effort to increase knowledge of HIV status through voluntary counseling and testing (VCT) programs for adults, provider initiated testing in clinical settings and HIV screening of pregnant women attending antenatal clinics (ANC). PEPFAR alone has supported 57 million HIV tests as part of VCT and ANC programs during its first five years, from 2004 through 2008. The total volume of HIV testing is likely to continue to increase over the coming decade, making the current estimate of 132 million annual HIV tests conservative.

In addition, there are an estimated 81 million annual blood donations, including 53 million (65%) in developed countries and 28 million (35%) in developing countries. There are an estimated 20 million plasma donations annually, including 16 million (80%) in the United States and European Union and 4 million (20%) in other countries. While overlap is likely between the estimate number of persons tested for HIV infection and the estimated numbers of blood and plasma donations, these two major compartments will be considered additive for the purpose of this exercise.

These 233 million annual HIV testing opportunities result in a very large potential market for acute HIV infection testing. Over a five-year period, more than one billion HIV tests are performed. The potential for acute HIV testing in the various segments of this market are discussed below.

BLOOD AND PLASMA DONATION

Assuring a supply of blood and blood product safe from HIV transmission is a critical global priority. In developed countries the desire and need to maximally reduce the risk of HIV transmission has meant that blood units are screened with a nucleic acid amplification test (NAAT), almost always using

Figure 4.2 PEPFAR HIV testing in VCT and PMTCT programs (2004-08)

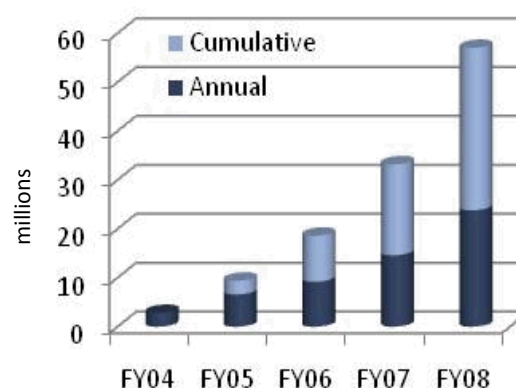


Table 4.2 PEPFAR HIV testing in VCT and PMTCT programs (2004-08)

FY04	2.8M
FY05	6.6M
FY06	9.2M
FY07	14.6M
FY08	23.8M
TOTAL	57.0M

Table 4.3 Estimated global blood and plasma donations per year (2006-07)

Blood	Developed countries	52.7M
	Developing countries	28.3M
	TOTAL	81.0M
Plasma	United States & EU	16.1M
	Other	4.5M
	TOTAL	20.6 M
TOTAL		101.6 M

a pooling process to reduce costs. Currently, NAAT or antigen testing is performed for a very large proportion of the 53 million blood donations and 16 million plasma collections that occur annually in developed countries. Most experts doubt that there will be room in this market for anything but the most sensitive diagnostic assay, currently NAAT. While a less-costly test would be desirable, such an assay would need to perform as well as current state-of-the-art NAAT assays.

In developing countries, there is additional potential demand for acute infection testing for the estimated 28 million blood donations that take place in these resource-limited settings. Some developing countries (e.g., Thailand and Brazil) do now use Ag/Ab screening and NAAT testing. However, it is challenging to determine what proportion of blood units are screened with such tests. In many developing country settings, the need to improve the quality of more basic blood banking procedures and comprehensive antibody testing continues.

DIAGNOSTIC HIV TESTING FOR ADULTS

Expert opinions varied widely on the appropriate use of acute HIV testing in adult diagnostic test settings. Given the large number of persons tested each year, hypothetically, even if only 5-20% of opportunities included a test for acute infection (virus or antigen), this results in seven to 26 million tests per year.

Table 4.4. Estimated number of acute diagnostic tests per year as proportion of total HIV testing, by region

Region	Estimated number of HIV tests (2006-7)	Number of Acute diagnostic tests as proportion of total HIV testing		
		5%	10%	20%
Africa	18.6 M	0.9M	1.9M	3.7M
Americas	38.3 M	1.9M	3.8M	7.7M
Asia	29.0 M	1.5M	2.9M	5.8M
Europe	41.3 M	2.1M	4.1M	8.3M
Rest of the world	4.9 M	0.3M	0.5M	1.0M
TOTAL	132.1 M	6.6M	13.2M	26.4M

However, the development of fourth generation combination antigen/antibody assays is quickly changing the market landscape. These assays that detect HIV infection approximately 16 to 20 days following the transmission event allow for prompt HIV diagnosis, including acute infection (due to p24 antigen detection) about six to eight days before the appearance of HIV antibodies detected on third generation assays. As discussed in Chapter 3, competition on the European HIV diagnostic market has driven the development of several new fourth generation assays. No such assay is currently licensed for use in the United States by the (FDA). There is now a simple, rapid, point-of-care fourth generation assay on the market (Inverness Determine) allowing for use in any clinical setting, including in developing countries. While NAAT does offer the ability to diagnose HIV infection approximately 11 days following

transmission, this is only about five days before detection on fourth generation combination assays. Such a narrow period substantially lessens the diagnostic advantage of NAAT testing for early diagnosis.

Clearly, the current large market described above is driving innovation and new, improved HIV diagnostic tests. Most experts felt that the inclusion of p24 antigen in fourth generation assays was driven by the desire to diagnose HIV infection as soon as possible after transmission to allow for proper diagnosis of infection status, rather than to identify persons in the pre-antibody acute period for special interventions. Indeed, many fourth generation assays do not provide a separate indication of the presence of antibody and/or antigen.

In developed countries, cost-effectiveness studies of the increased diagnostic benefit of NAAT testing of HIV-antibody-negative specimens indicate that such testing is very expensive and generally not cost effective. Many HIV prevention experts recommend that the increased investment would be better spent on expanded antibody testing of more people rather than searching for acute infection among those found to be HIV-antibody negative. In the United States, the state of North Carolina routinely uses pooled NAAT testing for early diagnosis; however, when fourth generation assays eventually are introduced, the additional benefit of NAAT testing will be markedly diminished.

EARLY INFANT DIAGNOSIS

The accurate diagnosis of HIV infection in children born to HIV-infected women is a high priority to assure prompt and appropriate HIV care and treatment. The presence of maternal antibodies necessitates viral (nucleic acid or antigen) testing during the first 18 months of life. More than one million HIV-positive women give birth annually, resulting in approximately 350,000 perinatally infected children.⁶⁶ Of these, the vast majority are in developing countries, with 90% in Africa.⁶⁷ The drive for innovation for improved, less-expensive p24 antigen and other virus assays for infant diagnosis has come mainly from the public sector. As described in Chapter 3, recent initiatives from the US NIH and the Gates Foundation have stimulated the development of improved infant diagnostics.

HIV VACCINE TRIAL PARTICIPANTS

Recipients of HIV vaccines in trials may become seropositive on current antibody assays because of the HIV antigens included in the vaccine. The need for a simple, rapid, inexpensive means of accurately diagnosing true HIV infection among HIV vaccine trial participants was the basis for a recent US NIH funding opportunity on assay development (see Chapter 3). The current, relative small market for such testing has not stimulated commercial development. However, if and when there is a safe and effective preventive HIV vaccine available for programmatic use, the demand for virologic assays to diagnose true HIV infection will be great. It is unlikely that an HIV vaccine will be available for programmatic use in the next 10 years.

MONITORING OF PERSONS ON ART

As described in Chapter 3, there has been recent innovation in the development of a virologic test that can be used to detect HIV viremia above a certain threshold (e.g., 1000 or 5000 copies/mL) for use in ART monitoring in resource limited settings. Such a test could be used to signal failure of an ART

regimen or non-adherence and signal the need for a switch to another regimen or to enhance adherence measures. The developing world is approaching 4 million persons on ART,⁶⁷ so a simple, inexpensive assay that could detect virologic treatment failure could have a potentially large market.

RESEARCH APPLICATIONS

The study of acute HIV infection is a high research priority in order to better understand HIV transmission and the early immune response in order to develop HIV vaccines and other improved interventions. The US NIH-supported CHAVI has supported a large research network and studies to identify acutely infected persons. NAAT testing of pooled specimens from HIV-antibody negative persons identified among at-risk populations has been the primary means of identifying acutely infected persons. It is expected that such research settings would continue to use state-of-the-art diagnostics and would not, on their own, drive market demand for new assays.

The current five-year total of more than *one billion* HIV testing opportunities creates enormous demand for improved HIV clinical diagnostics; it is likely that these numbers will increase over the next decade. This demand for improved, earlier HIV clinical diagnosis has driven the development of fourth generation antigen/antibody assays that have the potential of diagnosing HIV infection approximately 16 days following transmission and only about five days after the appearance of HIV RNA as detected by existing state-of-the-art nucleic acid-based tests. Consequently, a substantial part of what has been classified as acute HIV diagnosis will become standard testing where these new assays are available and used routinely. Market forces are driving the development of a wide variety of new assays. Public sector support for assay development is ongoing and will be required to develop simple, low-cost, point-of-care assays for use in developing countries for early infant diagnosis and the diagnosis of acute infection in adults to allow for early interventions.

Conclusions

There is a striking difference between the market for assays to estimate HIV incidence and assays to detect acute HIV infection. HIV incidence assays are used primarily for epidemiologic surveillance, programs monitoring and the assessment of specific population groups. Moreover, the current paradigm of HIV incidence assay use involves the testing of only the small proportion of specimens previously identified as HIV-seropositive on standard HIV antibody assays. Consequently, the market demand for HIV incidence assays is small, most likely less than one million specimens to be tested over a 5-year period. Not surprisingly, there has been very limited commercial interest in developing HIV incidence assays. Almost all activity on developing new assays has been by scientists at governmental agencies and academic institutions. While a number of commercially available HIV antibody assays have been modified for use in incidence estimation, only one novel HIV incidence assay, the BED EIA, which was developed at CDC, has been made commercially available.

The field of HIV incidence determination would be substantially altered by the development of an assay that determines both HIV-antibody-positivity as well as the recent status of the antibody response. Such an assay is under development at CDC. If this type of “game changing” assay is sensitive, specific (especially for recent infection status) for all HIV-1 subtypes and affordable, it could possibly result in a sharp increase in the demand for an incidence assay by perhaps 10-fold to six to 12 million specimens tested over five years. Meanwhile, the field of assay-based HIV incidence determination is currently moving toward the use of multi-test Recent Infection Testing Algorithms (RITAs).⁶

The market for assays to detect acute HIV infection (i.e., HIV nucleic acids or antigens) is robust. The market is driven by the need to screen the blood supply with the most sensitive assays available and the desire to diagnose HIV-infected individuals as early as possible following infection. There are an estimated 100 million units per year tested for blood donation or plasma collection. An additional 132 million specimens are tested for HIV diagnostic purposes each year. This results in an estimated 232 million annual HIV testing opportunities, or more than one billion over five years. Consequently, there is a very large potential market for acute infection testing. The European market, in particular, has driven the development of at least 12 combined HIV p24 antigen and antibody assays, termed fourth generation EIAs. The demand for improved, more sensitive assays is driven by the European regulatory process that periodically evaluates approved assays and removes from the market assays with poor performance. In the United States, FDA approved assays can remain on the market long after they have been eclipsed by newer, more sensitive assays. However, some assay manufacturers are pursuing the registration of fourth generation, combined antigen/antibody assays in the United States.

In developed countries, it is likely that fourth generation, combined HIV antigen/antibody assays will increasingly become the standard of diagnostic testing. However, in resource-limited, developing countries, where the burden of the HIV epidemic is greatest, the higher cost of these new fourth generation assays will likely limit their penetration into the market until prices come down. There are a number of new technologies under development, some funded by governments and foundations, that

might yield lower-cost assays suitable for low-tech, point-of-care testing. The US NIH recently funded three companies to advance the development of rapid point-of-care assays for use in infant diagnosis in developing countries and the identification of HIV infection among HIV vaccine recipients who may be antibody-positive due to the response to the vaccine.

Several assays to detect p24 antigen have been available for many years. More recently, Inverness marketed a rapid test (Determine HIV-1/2 Ag/Ab Combo) that detects p24 antigen and antibody separately on a test strip. CDC scientists are developing a p24 assay in a lateral flow format. Such assays, along with other new viral detection technologies, can play an important role in addressing the pressing need of early infant diagnosis for children born to HIV-infected mothers. The demand for infant HIV diagnosis is driven largely from developing countries, especially from Africa.

For the screening of blood and plasma donations in developed countries, NAAT testing will likely remain the standard. There is limited market potential for newer tests, unless they perform as well as current NAAT tests. In the developing world, where there is a diversity of testing quality, the market potential for improved, inexpensive antigen or NAAT tests is substantial.

As we approach the 30th anniversary of the emergence of the HIV/AIDS epidemic, the need for improved methods to accurately determine HIV incidence is greater than ever. A greater effort is needed to develop and evaluate HIV incidence assays in order to more effectively monitor the epidemic and better target prevention and care interventions. For the developing world, where the HIV burden is the greatest, there is a pressing need to make available acute infection assays affordable and usable in resource constrained settings.

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