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HIV Testing Updates and Challenges: When Regulatory Caution and Public Health Imperatives Collide

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Abstract Numerous improvements in HIV testing technology led recently to the first revision of recommendations for diagnostic laboratory testing in the USA in 25 years. Developments in HIV testing continue to produce tests that identify HIV infection earlier with faster turnaround times for test results. These play an important role in identifying HIV infection during the highly infectious acute phase, which has implication for both patient management and public health interventions to control the spread of HIV. Access to these developments, however, is often delayed by the regulatory apparatus for approval and oversight of HIV testing in the USA. This article summarizes recent developments in HIV diagnostic testing technology, outlines their implications for clinical management and public health, describes current systems of regulatory oversight for HIV testing in the USA, and proposes alternatives that could expedite access to improved tests as they become available.

Keywords HIV tests · HIV diagnosis · Acute HIV infection · FDA regulation

Introduction

On June 27, 2014, the Centers for Disease Control and Prevention (CDC) issued updated testing recommendations for the diagnosis of human immunodeficiency virus (HIV) infection [1••]. These represent not only the first update in the HIV diagnostic algorithm in the USA in 25 years but also the first time the recommendations for diagnostic testing have diverged completely from those for screening blood donations: none of the tests suitable for the new diagnostic algorithm are licensed for donor screening. It also marks the start of the kind of three-legged race involving technologic advances, clinical and public health imperatives, and regulatory caution that has characterized progress with HIV diagnostics in the USA for more than two decades.

Advances in Technology

Tables 1 and 2 list HIV diagnostic immunoassays approved by the Food and Drug Administration (FDA) as of December, 2014. Profound changes in testing technology have revolutionized laboratory testing for HIV, starting with the introduction of rapid assays for HIV antibody beginning in 2002 [2] and their subsequent eligibility for waived status under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) [3]. These point-of-care tests for immunoglobulin (Ig) G antibodies against HIV dramatically improved receipt of test results by decreasing turnaround time [4]. At the time the rapid tests were introduced, their performance was equivalent to or better than that of conventional HIV assays in widespread use [5]. Until 2007, the conventional assays used in the USA consisted of primarily indirect (second generation) enzyme immunoassays (EIAs) (HIV antigen in the solid phase to capture HIV antibody, and anti-IgG conjugated to the enzymatic detection marker in the liquid phase). In the presence of HIV antibodies, the enzymatic reaction produces a color change (measured as optical density), but second-generation EIAs are reactive with only IgG antibodies. The first third-generation EIA to employ the antigen sandwich technique (HIV antigen in the solid phase to capture HIV antibodies and HIV antigen, capable of binding to both IgM and IgG antibodies, conjugated to the enzymatic detection marker in

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Table 1 FDA-approved laboratory HIV immunoassays

Test	Markers used for detection	Analytes detected	Generation
Enzyme immunoassays			
Avioq HIV-1 Microelisa System	Viral lysate, native gp160	IgG antibodies	Second
Bio-Rad GS HIV-1/2 PLUS O	Recombinant p24, gp160, HIV-2 gp36, synthetic group O peptide	IgG and IgM antibodies	Third
Bio-Rad GS HIV Combo Ag/Ab EIA	Synthetic gp41, recombinant gp160, HIV-2 gp36, synthetic group O peptide, p24 monoclonal antibodies	IgG and IgM antibodies p24 antigen	Fourth
Chemiluminescent assays			
Abbott Architect HIV Ag/Ab Combo	Synthetic and recombinant gp41 and HIV-2 gp36, group O peptide, p24 monoclonal antibodies	IgG and IgM antibodies P24 antigen	Fourth
Ortho Vitros Anti-HIV 1+2	Recombinant p24, gp41, gp41/120, HIV-2 gp36	IgG and IgM antibodies	Third
Siemens Advia Centaur HIV 1/O/2	Recombinant gp41/120, p24, HIV-2 gp36, synthetic group O peptide	IgG and IgM antibodies	Third

the liquid phase) received approval from the FDA in 1992. However, third-generation assays did not come into widespread use for diagnostic testing until the mid-2000s when, simultaneously, manufacturers began to introduce chemiluminescent immunoassays (CIAs) [6–8]. CIAs employ particles coated with HIV antigen to capture HIV antibodies, and for detection, use HIV antigen conjugated to a luminescent chemical. In the presence of antibodies, reaction with the marker emits light, measured as relative light units. CIAs offer several advantages for clinical diagnostics: they require shorter incubation and reaction times, which can reduce testing time to less than an hour, and they are suitable for use in random access analyzers intended to process specimens one at a time rather than in batches. Thus, rapid results are possible from assays designed for automation and high throughput. Third-generation EIAs and CIAs all incorporate antigens for specific detection of antibodies against HIV-1, HIV-2, and HIV-1 group O. Fourth-generation EIAs and CIAs (termed antigen/antibody combo assays) add concurrent detection of HIV-1 p24 antigen [9, 10]. As of October 2014, the FDA-approved conventional antigen/antibody combination immunoassays do not identify which component (that is, antigen or antibody) causes a positive reaction. However, a new assay based on a bead multiplexing technique has recently been submitted for FDA approval [11]. The assay uses magnetic beads coated with antigen or antibody and different fluorescent markers. When exposed to lasers (in a manner analogous to flow cytometry), identification of specific beads allows determination of which component (HIV-1 antibody, HIV-2 antibody, or p24 antigen) causes reactivity.

Most single-use rapid HIV tests are based on second-generation principles, using HIV antigens embedded in either a lateral flow strip (immuno-chromatography) or on a flow-through membrane (immuno-concentration) to capture antibodies. Antibody detection is accomplished by colloidal gold conjugated to protein A, which binds with high affinity to human IgG and produces a color change that is interpreted visually [12]. The sensitivity of lateral flow rapid tests with

Table 2 FDA-approved rapid and point-of-care HIV tests

Test[a]	Specimen types	Markers used for detection	CLIA category	Generation
OraQuick Advance Rapid HIV-1/2 Antibody Test	Oral fluid, whole blood; plasma	gp41, gp36	Waived[b]; moderate complexity	Second
Reveal G3 Rapid HIV-1 Antibody Test	Serum, plasma	gp41, gp120	Moderate complexity	Second
Uni-Gold Recombigen HIV-1/2	Whole blood; serum, plasma	gp41, gp120, gp36	Waived; moderate complexity	Third
Multispot HIV-1/HIV-2 Rapid Test	Serum, plasma	gp41, gp36	Moderate complexity	Second
Alere Clearview HIV 1/2 Stat Pak	Whole blood; serum, plasma	gp41, gp120, gp36	Waived; moderate complexity	Second
Alere Clearview Complete HIV 1/2	Whole blood; serum, plasma	gp41, gp120, gp36	Waived; moderate complexity	Second
INSTI HIV-1 Antibody Test Kit	Whole blood; serum, plasma	gp41, gp36	Waived; moderate complexity	Second
Chembio DPP HIV 1/2 Assay	Oral fluid, whole blood, serum, plasma	gp 41, gp120, gp36	Waived; moderate complexity	Second
Alere Determine HIV 1/2 Ag/Ab Combo	Whole blood, serum, plasma	gp41, gp120, gp36; p24 antibodies	Waived; moderate complexity	Fourth
Geenius HIV 1/2 Supplemental Assay	Whole blood, serum, plasma	p24, p31, gp41, gp 160, gp36, gp140	Moderate complexity	Second

[a] Tests are listed in the order in which they received FDA approval

[b] Waived status applies only when tests are used with direct, unprocessed specimens (whole blood or oral fluid)

plasma specimens during seroconversion is similar to that of the Western blot, a first-generation assay for IgG antibodies that uses capture antigens derived from lysate of whole HIV [13, 14••]. Flow-through rapid assays detect antibodies several days sooner. Three rapid tests that employ novel methods recently received FDA approval. The Alere Determine HIV-1/2 Combo Ag/Ab, a lateral flow assay (approved December 2013) employs the antigen sandwich technique with a colloidal selenium conjugate to detect HIV-1 and HIV-2 antibodies sooner after seroconversion and, at a separate location on the test strip, p24 antigen [15]. The DPP HIV1/2 was approved in December 2012 for use with serum, plasma, whole blood, and oral fluid [16]. Specimen is added to the device and flows from one direction across the test strip, onto which one or more antigens have been adsorbed; the detection agent (colloidal gold conjugated to protein A) is added to flow from a perpendicular direction. This principle appears to be more useful for multiplex testing for different antibodies on the same strip and is utilized by another HIV-1/HIV-2 antibody test, the Geenius HIV 1/2 Supplemental Assay, which incorporates four separate HIV-1 antigens (p24, p31, gp41, and gp 160) and two HIV-2 antigens (gp36 and gp140) to differentiate HIV-1 from HIV-2 antibodies with whole blood, serum, or plasma specimens. This test uses an automated reader and software that provides interpretation of results based on the presence and intensity of the bands and received FDA approval as a supplemental test in October 2014 [17].

Rapid tests suitable for use at point of care can utilize finger-stick, venous whole blood, or oral fluid specimens for testing. The concentration of antibodies in these alternative specimens is lower than that in serum or plasma. Oral fluid tests initially generated considerable enthusiasm because specimen collection was non-invasive and testing could easily be performed in a variety of settings where blood collection might not be feasible. This enthusiasm has waned, however, because of sporadic instances of excessive false-positive results [18, 19] and more significantly false-negative result in persons on antiretroviral therapy (ART) [20] and during the early stages of HIV infection [21•, 22, 23•]. Reports have also begun to surface of significant delays in detection of seroconversion in some persons receiving pre-exposure prophylaxis (PrEP; CDC, unpublished data), which contravenes the use of oral fluid for the initial or follow-up testing required for the initiation or continuation of PrEP.

Five HIV-1 quantitative (viral load) nucleic acid amplification tests (NAATs) are available to assess patient prognosis and monitor response to antiretroviral therapy (Table 3), but only one qualitative HIV-1 RNA assay is FDA-approved for diagnosis [24]. Currently available HIV-1 RNA assays are expensive and require a sophisticated laboratory to perform, but technologic advancements in HIV testing may expand the use of HIV-1 RNA assays for HIV diagnosis in the future. Two simplified rapid NAATs are now commercially available outside the USA: one is qualitative or semi-quantitative [25] and the other is a quantitative viral load assay [26]. Both are suitable for near-patient diagnosis in non-laboratory settings, but neither has been submitted for FDA approval.

Clinical and Public Health Implications

Newer generations of immunoassays improve sensitivity for detecting early HIV-1 infection and narrow the interval between the time of infection and its detection (Fig. 1), allowing earlier diagnosis of HIV infection. This confers utility for both clinical practice and for prevention and control as the importance of detecting acute HIV infection has become increasingly apparent [27, 28, 29•, 30]. Treatment of acute and early HIV-1 infection with combination ART improves laboratory markers of disease progression [31, 32]. Limited data also suggest that treatment of acute HIV-1 infection might decrease the clinical severity of acute disease, lower the viral set point, slow disease progression in the event therapy is stopped, reduce the size of the viral reservoir and decrease the rate of viral mutation by suppressing viral replication and preserving immune function [33–37]. Acute HIV-1 infection plays a disproportionate role in sustaining epidemic spread because very high levels of virus in blood and genital secretions increase infectiousness substantially compared to the long asymptomatic phase of HIV [30, 38]. The acute stage of infection can be nearly 30 times more infectious (per sex act) than those during the long asymptomatic phase [39], and in some populations, acute HIV infection accounts for as much as 50 % of all onward transmission of HIV infection [40]. Initiating treatment during acute infection can reduce the risk of HIV-1 transmission substantially [29•, 34, 41]. Since March 2012, the US Department of Health and Human Services Panel on Antiretroviral Guidelines for Adults and Adolescents has recommended initiation of ART for all persons with HIV-1 infection to reduce the risk of disease progression and to prevent HIV transmission [34].

HIV-1 Western blot has long been the gold standard for confirmation after a reactive initial immunoassay [42], but its shortcomings (as a test that can confirm only the presence of IgG antibodies) have become increasingly evident. Numerous studies document that high levels of HIV-1 RNA are present in persons with acute HIV infections that are detected by sensitive immunoassays but negative or indeterminate by Western blot [43•, 44–49]. Because of cross-reactivity, the HIV-1 Western blot was interpreted as positive for HIV-1 in 46 to 85 % of specimens from persons found to be infected with HIV-2, resulting in incorrect or delayed diagnosis [50–52]. Although HIV-2 remains uncommon in the USA, accurate diagnosis of HIV-2 is clinically important because some antiretroviral agents effective against HIV-1 (including non-nucleoside reverse transcriptase inhibitors and some protease inhibitors) are not effective against HIV-2 [53, 54].

Table 3 FDA-approved quantitative viral load assays and specimen requirements

Test and manufacturer	Amplification method; target	Anticoagulant	Plasma volume[a]	Range (copies/ml)
Amplicor HIV-1 monitor version 1.5 (Roche Diagnostics, Indianapolis, IN)	RT-PCR; gag gene	EDTA, ACD		
Standard			200 μl	400–750,000
Ultrasensitive			500 μl	50–100,000
Cobas AmpliPrep/Cobas TaqMan HIV-1 Version 2.0 (Roche Diagnostics, Indianapolis, IN)	Real-time RT-PCR; LTR, gag gene	EDTA	1 ml	20–10,000,000
RealTime HIV-1 (Abbott Molecular, Des Plaines, IL)	Real-time RT-PCR; integrase gene	EDTA, ACD	1 ml	40–10,000,000
Versant HIV-1 RNA 3.0 (bDNA) (Siemens Healthcare Diagnostics, Tarrytown, NY)	bDNA; pol gene	EDTA, ACD	1 ml	75–500,000
NucliSens HIV-1 QT (BioMérieux, Inc., Durham, NC)	NASBA; gag gene	EDTA, ACD, heparin	1 ml	176–3,470,000

[a] For tests that use an automated extraction instrument (AmpliPrep and RealTime), the listed specimen volume is the volume of sample that is loaded on the instrument, which is greater than the actual volume of specimen used for the extraction

HIV-1 RNA testing after a reactive fourth-generation immunoassay can confirm a higher proportion of HIV diagnoses than antibody-based assays such as Western blot or the Multispot HIV-1/HIV-2 differentiation assay (97.9 % versus 94.6 and 93.5 %, respectively, in one study of 664 HIV-infected persons [55•]). Testing only for RNA, however, is insufficient to confirm the diagnosis of HIV infection in all cases: HIV-1 RNA was undetectable in 2 to 4 % of specimens that were reactive on immunoassays and positive on HIV-1 Western blot [13, 48, 55•, 56, 57]. Without concurrent antibody testing, the presence of HIV-1 RNA does not distinguish acute from established HIV infection. The updated CDC testing recommendations strive for parsimony by first testing all specimens initially reactive on a fourth-generation combo immunoassay with an HIV-1/HIV-2 antibody differentiation assay, which identifies the majority of HIV infections (those that are antibody-positive) and the occasional HIV-2 infection. The essential feature of the updated algorithm is the subsequent testing for HIV-1 RNA, those specimens negative for IgG antibodies in order to identify acute HIV infections [1]. Currently, only the Aptima HIV-1 RNA Qualitative Assay, FDA-approved for HIV diagnosis, can be used by laboratories for reflex testing as part of the updated algorithm. However, because it is available in far fewer laboratories than quantitative HIV-1 (viral load) RNA assays, clinicians can order a viral load test to facilitate prompt diagnosis of acute HIV infection when faced with discordant screening and supplemental antibody test results and to differentiate acute HIV-1 infection from false-positive initial immunoassay results [43].

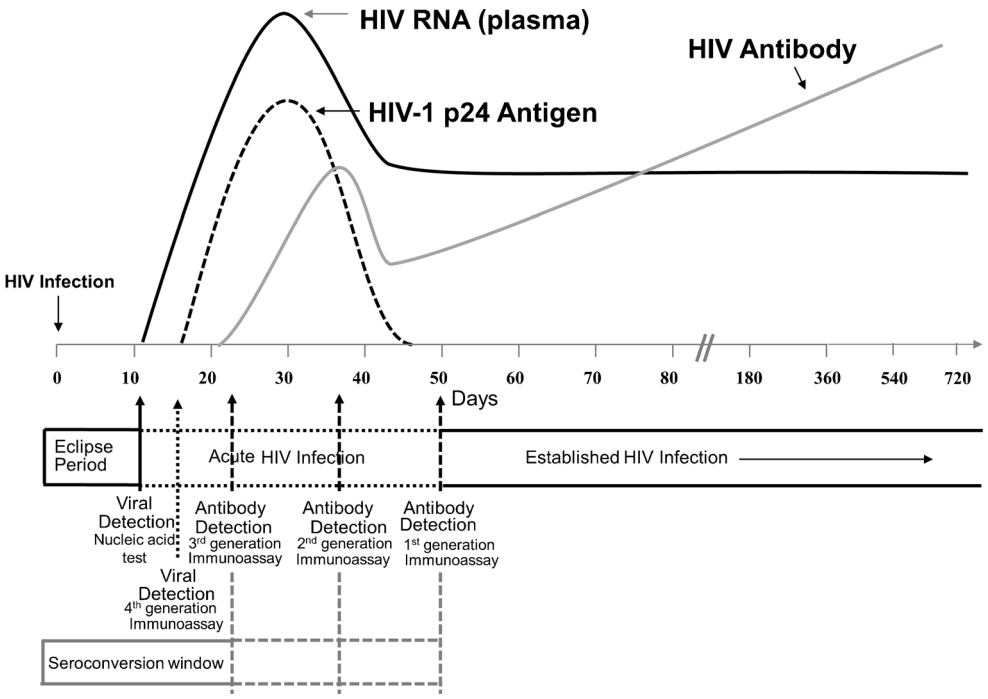

Fig. 1 Sequence of appearance of laboratory markers for HIV-1 infection. Note. Units for vertical axis are not noted because their magnitude differs for RNA, p24 antigen, and antibody. Modified from MP Busch, GA Satten (1997) [94] with updated data from Fiebig (2003) [95], Owen (2008) [13], and Masciotra (2011, 2013) [14••, 85]

Regulatory Challenges

HIV laboratory diagnostics are governed by three mechanisms of oversight. The FDA reviews and classifies laboratory assays and regulates their sale and distribution subject to the statutory and regulatory framework set forth in the Food, Drug, and Cosmetic Act. The Centers for Medicare and Medicaid Services regulates laboratories performing diagnostic tests under the authority of CLIA. CDC establishes case definitions for HIV and issues recommendations for using tests in combination for accurate HIV diagnosis. One inadvertent consequence derives from this extensive oversight: the perfect sometimes becomes the enemy of the good, delaying introduction of significant technologic improvements. For example, fourth-generation HIV antigen/antibody combination assays were introduced in Europe more than 10 years before they became available in the USA [58, 59]. During that 10-year hiatus, as many as 10 to 20 % of persons in some high-incidence populations who were infected with HIV (and highly infectious) at the time they were tested received false-negative HIV test results [21•, 22, 48, 60].

Congress and the FDA have developed a system of device classification to facilitate different levels of oversight for devices with different risk profiles [61]. Class I includes devices with the lowest risk. Most diagnostic tests typically are class II devices that pose a moderate risk to patients or users and are subject to the premarket notification [510(k)] pathway. Devices that have substantial importance for prevention of impairment of health or a potential unreasonable risk of illness or injury (whether by themselves or because of the way the test results are used for medical decision-making) are class III (high risk) devices. HIV diagnostics, because of their historical use for screening blood donations, are class III. As such, they are subject to the highest level of review, premarket approval application (PMA). Devices intended for blood screening require a further biologic license application (BLA) and are subject to additional controls and lot-release testing by the FDA. By statute, application fees for 510(k) clearance are specified as 2 % of the application fee for PMA approval ($5018 and $250,895, respectively, for fiscal year 2015 [62]).

The approval process for an HIV diagnostic test is time-consuming and expensive. The FDA does not publish requirements for the number of specimens that must be tested or other essential standards that HIV diagnostic tests must meet, unlike the Common Technical Specifications prescribed by the European Union [63]. Manufacturers must glean these requirements from the package inserts or the summaries of safety and effectiveness of other products that have been approved previously and then submit proposals for their planned application, specifying the intended use of the device and the clinical trials that will be used to support the application. At a pre-submission meeting (usually scheduled within 90 days after it is requested), the FDA provides feedback on the adequacy of the proposal and indicates any additional data that might be necessary (for example, for the number of specimens that must be collected prospectively). These specifications often change. Clinical trials must then be conducted with each type of specimen (e.g., serum, plasma, or whole blood) that will be used with the assay. Usually, 7000 to 9000 specimens are tested (Table 4), most of which are collected prospectively from populations in the USA over 1 to 2 years at an approximate cost of $1000 per specimen. For tests that use specimens that cannot be stored (e.g., whole blood or oral fluid), all specimens must be collected prospectively and tested immediately. Once trials are complete, the manufacturer then prepares a PMA application, which specifies the intended use of the assay and contains a complete record of the studies performed to demonstrate its safety and effectiveness for that use and detailed information on how the device is designed and manufactured [61]. The application is reviewed at FDA by a group of regulatory scientists, including statisticians who review the study design and often perform their own independent analysis of the raw data. This review usually takes 6 months or more, and then clinical trial sites are audited for compliance with good laboratory practices; manufacturers' facilities are also inspected to assure compliance with the Quality Systems Regulation. This process, from the pre-submission meeting until ultimate approval, typically requires 2 to 3 years. The entire approval process must be repeated for even minor modifications to the assay procedure or reagents or change in the intended use (for example, testing a different specimen matrix, such as dried blood spots, or as recently occurred, for using the Multispot HIV-1/HIV-2 differentiation assay as supplemental test in addition to its previously approved intended use as a screening test).

Requirements for approval can sometimes seem arbitrary or variable. For example, HIV assays are required to demonstrate, as a condition of approval, sensitivity for the extremely rare HIV-1 group O variant (a requirement that was suspended for rapid HIV assays) [64], but ability to detect the more prevalent (although still uncommon) HIV-2 is optional. FDA and the Blood Products Advisory Committee specify that, for HIV tests, the lower bound of the 95 % confidence interval (CI) must be at least 99 % for laboratory assays, 98 % for single-use rapid tests, and 95 % for home-use HIV tests. This tends to increase the size of clinical trials in order to achieve narrow intervals. Moreover, in 2012, the FDA approved an HIV test for in-home use that demonstrated a sensitivity in clinical trials of only 91.7 % (95 % CI 84.2 %, 96.3 %) [65]. This approval was based on a mathematical model of the theoretical benefits for public health that predicted, somewhat implausibly, that the home test would identify 45,000 new HIV infections during its first year of use [66]. (All HIV testing activities in the USA combined identified an average of 47,000 new HIV diagnoses per year from 2008 to 2012 [67].)

Table 4 Typical clinical trial requirements for FDA approval of HIV assays[a]

Parameter	Number of specimens
Sensitivity	900–1000 known HIV-1, including 100 from persons with stage 3 (AIDS) and 200–300 from non-B subtypes, group O, group N 200–300 known HIV-2
Seroconversion panels	20–30
Specificity	6000–7000, including a minimum of 1000 from low-risk populations, 600–700 from high-risk populations, 500 from HIV-2-endemic area
Potentially cross-reacting unrelated medical conditions	200–300
Total specimens	7300–8600

[a] Information derived from performance characteristics reported in package inserts of HIV assays recently approved by the Food and Drug Administration

Once approved, tests remain commercially available even when they are superseded by assays with superior performance. Manufacturers are reluctant to submit updated versions of assays for US regulatory review as quickly as they do in Europe because it is expensive and time-consuming to do so. Because no mechanism exists for periodic comparative evaluation of assays on the same challenge panels, most clinicians and laboratory scientists are unaware that the performance of FDA-approved tests can differ substantially relative to each other [68].

Although most diagnostic devices fall under the purview of the Center for Devices and Radiological Health (CDRH), premarket review of tests for infectious agents that involve the blood supply and for retroviral testing is performed within the Center for Biologics Evaluation and Research (CBER). However, categorization of device complexity (high complexity, moderate complexity, or waived, as required by CLIA) is performed by CDRH. Manufacturers must apply to CDRH for CLIA waiver after CBER approves the PMA application, and usually additional clinical trials are required for the waiver application. Most conventional laboratory HIV assays are categorized as high or moderate complexity under CLIA and thus subject to considerable regulatory oversight. Requirements for personnel qualifications, training, and proficiency testing are minimal for CLIA-waived tests, which are defined as tests so simple and accurate such that the likelihood of an erroneous result is negligible [69]. CLIA waiver of rapid HIV tests (the first waiver granted to a test for a communicable disease) caused considerable alarm among laboratory professionals [70]. This led to additional recommendations for promoting quality assurance procedures for waived tests [71] and subsequently revised FDA guidance that imposed additional requirements on manufacturers seeking waiver of in vitro diagnostic tests [72]. For HIV, these new requirements mandate additional clinical trials that must prospectively identify at least 30 HIV-positive persons who have not been previously diagnosed; no results from this testing can be disclosed to the trial participants. Given the low prevalence of new diagnoses at most testing sites, these trials require testing 1500 or more persons over 1 to 2 years at a cost of approximately $1.5 million, in addition to the clinical trials required for approval. Testing must be conducted by individuals with no laboratory background who have had no training in the use of the test (unlike the training provided in settings where waived tests are likely to be used). Results from the untrained users must be compared to those of an FDA-approved reference method performed in a laboratory. Before these new requirements were imposed in 2008, waiver studies could be conducted in less than 2 months at a cost of approximately $50,000: 100 untrained users tested a challenge panel of six specimens; their results were compared to the results from trained laboratory professionals conducting the same test on the same specimen panels.

The Three-Legged Race

Regulatory hurdles create challenges for practitioners. Several examples illustrate the problems this creates. CDC recommends HIV testing of newborns as soon as possible after birth when the mother's HIV status is unknown postpartum so that antiretroviral prophylaxis can be offered to HIV-exposed infants to reduce mother-to-child transmission [73]. However, no HIV tests are FDA-approved for use in children <2 years of age, and, under CLIA, laboratories cannot provide patient results from a test for an indication that is not specified in the manufacturer's product insert without first conducting an extensive performance verification [74]. Similarly, quantitative HIV viral load tests are performed by many laboratories and could provide prompt information with both diagnostic and clinical utility for patients with acute HIV infection [43]. However, these perfectly reliable tests are not FDA-approved for diagnosis. Although clinicians can order a viral load test for this purpose, laboratories cannot perform them off-label reflexively as part of the updated diagnostic algorithm without first conducting an extensive verification comparing their performance to that of the qualitative RNA assay approved for diagnosis. Finally, dried blood spots have proven to be an ideal specimen type that is easy to obtain in settings where phlebotomy is not feasible or when transport of venipuncture specimens is impractical. They produce accurate results with antigen and antibody immunoassays and nucleic acid tests [75–78]. However, only suboptimal second-generation IgG-only

EIAs, the HIV-1 Western blot, and the indirect immunofluorescence assay are FDA-approved for use with dried blood spot specimens, which severely limits their utility with the updated diagnostic algorithm.

New developments also occur faster than evidence-based recommendations can be written. Ample evidence demonstrated that the FDA-approved Multispot HIV-1/HIV-2 differentiation assay provided results superior to those of the HIV-1 Western blot when used as a supplemental test [45, 52, 79–83]. However, because it was approved only for HIV antibody screening, it could not be recommended as a supplemental test in diagnostic algorithms until the manufacturer obtained FDA approval for this intended use [84]. The evidence for performance of the Alere Determine HIV 1/2 Combo rapid test and the Bio-Rad Geenius HIV 1/2 supplemental assay is not yet sufficient to warrant an immediate revision of CDC's 2014 updated testing algorithm, but their performance with reference panels suggest they are likely to represent pragmatic alternatives for a large number of laboratories [49, 85–87]. These laboratories will need to delay the use of these tests or rely on expert opinion while sufficient data accumulates to meet the strict evidentiary requirements necessary for an updated practice guideline [88]. Laboratories that screen for emergency organ donation face an additional dilemma. Because none of the tests recommended in CDC's updated diagnostic algorithm are licensed for screening blood or organ donations, the laboratories must maintain separate, archaic (but licensed) testing platforms to meet regulatory requirements.

The lag in the availability of improved HIV assays in the USA is disturbing. Alternatives exist to harmonize regulatory processes so that they do not impose barriers between assay development and marketing, assure that commercial kits are of high quality, and discourage the use of obsolete tests. In Europe, published common technical specifications provide explicit guidance to manufacturers on clinical trials required for approval [63], and numerous HIV assays (many developed by US scientists and manufacturers) are registered with regulatory agencies. Public health and other independent agencies conduct challenge studies every several years in which registered assays are evaluated against a panel of specimens selected to represent early seroconversion to HIV-1 subtype B, other subtypes of HIV-1 (including HIV-1 group O samples), and HIV-2. Assays are ranked on the basis of the total number of samples identified as reactive; results of these studies are published in the literature. Only the top-performing assays are recommended to laboratories for continued use [68, 89–91].

Conclusions

Experience with HIV has brought about numerous changes in the conduct of clinical trials (such as community advisory boards and surrogate endpoints) and new drug approval (including expanded access programs and expedited approval pathways). It is time for such innovation to encompass HIV diagnostics. Requirements for the size of clinical trials need to be published, reasonable and uniform. As was recently done for tuberculosis tests [92], reducing the class III high-risk designation of HIV diagnostics would allow manufacturers to use faster, more streamlined approval pathways. If HIV diagnostic tests were approved on the basis of their function—detection of HIV-1 and HIV-2 antibodies or detection of HIV-1 RNA, for example, instead of an intended use such as screening, supplemental testing, or monitoring—laboratories could use existing tests for new applications when evidence for their utility develops. At a minimum, laboratory experts should be free to use tests for any appropriate purpose whenever adequate evidence exists in the peer-reviewed literature, without waiting for extensive (and expensive) clinical trials and a separate FDA approval. Head-to-head comparison studies, such as those published by the CDC [13, 14••, 85], should serve as the basis for encouraging (or discouraging) the use of FDA-approved tests based on objective performance criteria.

Since approval of the home sample collection system for HIV testing in 1996, FDA has included public health benefit as part of their mandate to determine the safety and effectiveness of in vitro HIV diagnostics [93]. It is time to apply those same principles to a revision of both the FDA approval process and the CLIA oversight process so that advances in technology can expeditiously support the rapid progress that has accompanied the clinical and public health approaches to the diagnosis and management of HIV disease.

Compliance With Ethics Guidelines

Conflict of Interest Bernard M. Branson declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by the author.

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