HIV Diagnostics

Current Recommendations and Opportunities for Improvement

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KEYWORDS

• HIV testing • HIV antibody test • HIV diagnosis • HIV testing algorithm
• HIV viral load

KEY POINTS

- Instrumented laboratory human immunodeficiency virus (HIV) tests detect immunoglobulin G (IgG) and IgM antibodies and HIV-1 p24 antigen and are more sensitive during early infection.
- Rapid point-of-care HIV tests detect IgG antibodies and can be used with whole blood or oral fluid but are less sensitive during early infection.
- Recommended HIV testing begins with a sensitive p24 antigen-HIV-1/HIV-2 antibody combination immunoassay that can detect HIV as soon as 2 weeks after infection.
- An HIV-1 viral load test might be used after an initially reactive immunoassay to confirm infection and provide more clinically useful information than the recommended second antibody test.
- Early initiation of antiretroviral therapy and taking preexposure prophylaxis can alter the evolution of HIV biomarkers and reactivity of immunoassays and lead to ambiguous results.

INTRODUCTION

According to the Centers for Disease Control and Prevention (CDC), an estimated 1.1 million persons aged ≥13 years in the United States were living with human immunodeficiency virus (HIV) infection at the end of 2015, an estimate that has remained consistent since 2003.[1,2] The percentage unaware of their infection decreased from 25% in 2003 to 14.5% in 2015, where it has been relatively stable. The benefits of early diagnosis and immediate antiretroviral therapy (ART) for both improving health and preventing transmission are compelling. The CDC, the US Preventive Services Task Force, and professional medical associations recommend routine HIV screening

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and selective, periodic retesting, but as of 2017, only 46% of persons 18 to 64 reported ever having had an HIV test.[3–6]

HIV diagnostics are essential for both diagnosis and management of HIV infection. The laboratory diagnosis of HIV employs a sequence of tests with an algorithm for resolving discordant test results to maximize overall sensitivity and specificity.[7] In June 2014, the CDC issued updated testing recommendations for the diagnosis of HIV infection.[8] This update marked the first time tests recommended for HIV diagnosis diverged completely from those used for screening blood donations: none of the tests in the diagnostic algorithm are licensed for donor screening. Although clinicians often do not know which specific assays will be used when they order an HIV test, the tests differ in subtle ways with different implications depending on the reasons for testing. This article provides a brief review and history of HIV test development, describes technologies currently in use, current recommendations for which tests to use, discusses options for the choice of confirmatory tests, and mentions the potential effects of early antiretroviral treatment and PrEP on HIV test results.

VIROLOGY

HIV exists as 2 major viral species. HIV type 1 (HIV-1), identified first, is the more virulent of the 2 and responsible for most AIDS cases worldwide. HIV-2, first isolated in 1986, has properties similar to those of HIV-1, but is less pathogenic, differs in some of its antigenic components, and has a more limited geographic distribution.[9] The mature HIV virion consists of 2 copies of single-stranded RNA surrounded by structural proteins, a matrix shell, and lipid envelope. The RNA genome contains the *env*, *gag*, and *pol* genes, which encode envelope glycoproteins, structural proteins, and viral enzymes, respectively. The Western blot technique was used to separate the HIV-1 viral proteins by their molecular weight (**Fig. 1**). These proteins were later

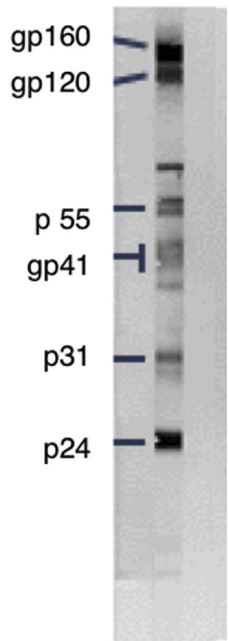

Fig. 1. Positive HIV-1 Western blot result.

related to HIV morphology (Fig. 2). The nomenclature of viral proteins indicates "gp" for glycoprotein or "p" for protein followed by a number representing its molecular weight. The major components of diagnostic utility for HIV-1 include envelope proteins (gp41, gp120, and their precursor, gp160), the core gene proteins (p55, p24, p17), and the polymerase (*pol*) gene proteins (p66, p51, p31). HIV-2 proteins are similar but differ somewhat in the molecular weight of the individual gene products (eg, p26 corresponds to p24; gp36 and gp105 correspond to gp41 and gp120).

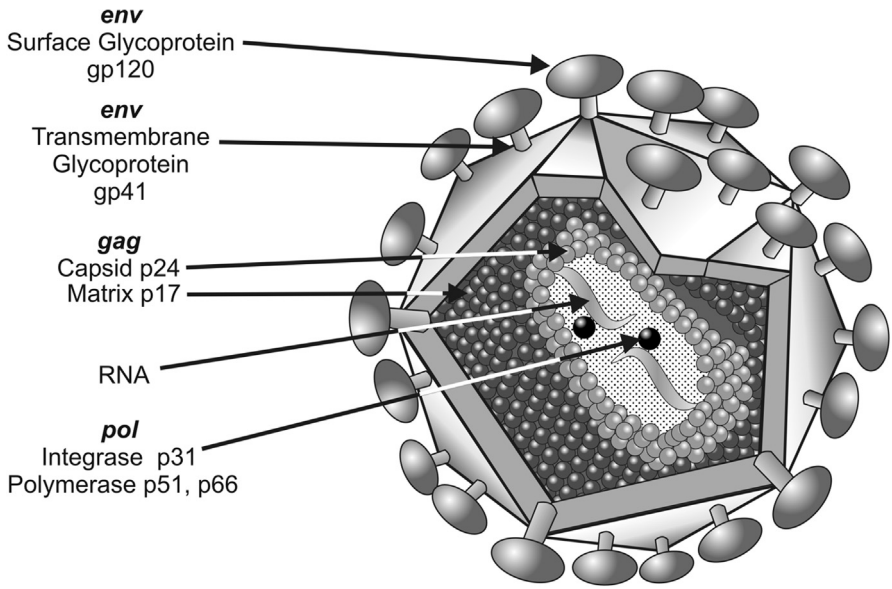

Fig. 2. Structure of the HIV-1 virion.

EVOLUTION OF HUMAN IMMUNODEFICIENCY VIRUS DIAGNOSTICS

After HIV-1 infection, HIV-1-specific markers appear in the blood in the following chronologic order: HIV-1 RNA, p24 antigen, HIV-1 immunoglobulin M (IgM) antibody, and HIV-1 IgG antibody (Fig. 3). Time from HIV acquisition to reactivity for an assay depends on which target is being detected, when that target can be detected after infection, concentration of the target in the specimen, the volume of specimen tested, and the test's analytical sensitivity. Understanding how these variables affect test results is essential for understanding the advantages and limitations of different types of tests for specific clinical situations.

Serologic tests for HIV have been grouped informally into generations based on the test's principle. Each subsequent generation led to a shorter false-negative window period between infection and detection. As tests other than enzyme immunoassays (EIAs) emerged, including point-of-care rapid tests, classification by generation became less clear cut, and an alternative taxonomy has been suggested.[10] This article refers to tests by generation as described in CDC's 2014 HIV laboratory testing recommendations (Box 1).[8]

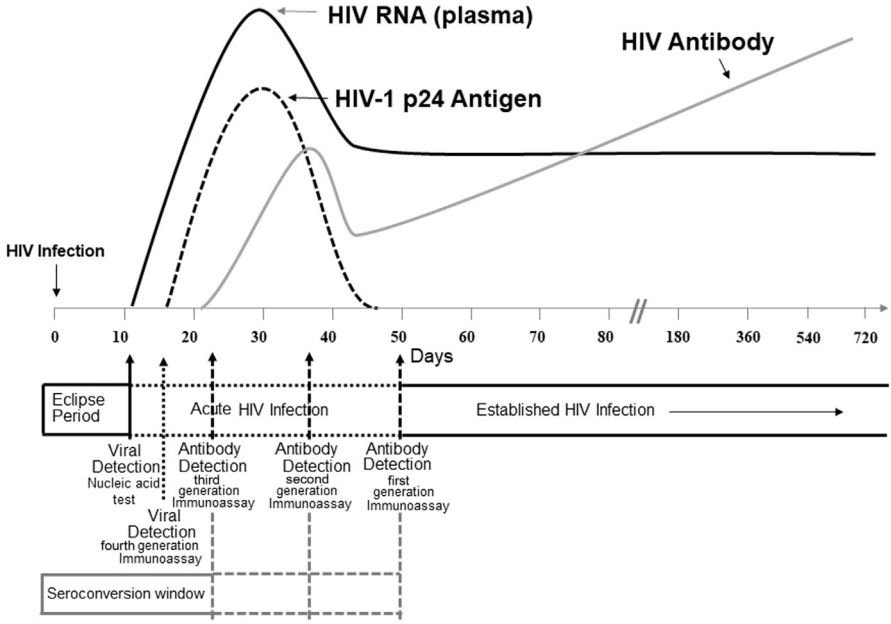

Fig. 3. Sequence of appearance of laboratory markers for HIV-1 infection. Units for vertical axis are not noted because their magnitude differs for RNA, p24 antigen, and antibody. (*Data from* Refs.[22,23,30])

Box 1
Human immunodeficiency virus immunoassay techniques

First generation

Antigens used to bind anti-HIV antibodies: lysate of whole HIV-1 viruses grown in cell culture

Format: indirect immunoassay using labeled anti-human IgG

Detects: IgG anti-HIV antibodies

Second generation

Antigens used to bind anti-HIV antibodies: synthetic peptide or recombinant viral proteins

Format: indirect immunoassay using labeled anti-human IgG or, in rapid tests, protein A, which binds to human IgG with high affinity

Detects: IgG anti-HIV antibodies

Advantages: eliminating cellular contaminants from viral lysates improves specificity by eliminating cross-reactivity with cellular proteins; design of specific antigenic epitopes improves sensitivity for HIV-1, group O, and HIV-2

Third generation

Antigens used to bind anti-HIV antibodies: synthetic peptide or recombinant viral proteins

Format: antigen sandwich (HIV antibodies in the specimen bind to HIV antigens on the assay substrate and then to HIV antigens conjugated to indicator molecules)

Detects: IgG and also IgM anti-HIV antibodies, which develop sooner

Advantage: becomes reactive earlier during seroconversion

Fourth generation

Antigens used to bind anti-HIV antibodies: synthetic peptide or recombinant viral proteins; also, contain monoclonal anti-p24 antibodies

Format: antigen sandwich

Detects: IgG and IgM anti-HIV antibodies, HIV-1 p24 antigen

Advantage: becomes reactive shortly before HIV-1 seroconversion

The earliest HIV EIAs used lysate of whole HIV-1 purified from cell cultures as the source of antigens to bind antibodies to HIV-1. Anti-human IgG conjugated to an enzyme would bind to HIV-1 antibodies, if present, and produce a color change when the enzyme's substrate was added. The viral lysate EIAs had high empirical sensitivity for HIV IgG antibodies but could remain negative for up to 12 weeks after infection before antibodies developed.[11] False-positive test results also occurred, associated with other infections, pregnancy, autoimmune disease, and unspecified conditions. Concerns about the potential for false-positive results from screening with a test with uncertain implications[12] led public health officials to endorse a 2-test strategy to maximize specificity. The HIV-1 Western blot also uses first-generation principles: whole viral lysate as the source of antigens and anti-IgG conjugated to an enzyme to bind to individual HIV proteins. In 1989, the Public Health Service established minimum criteria for a positive Western blot interpretation (presence of any 2 of the p24, gp41, and gp120/gp160 bands) and recommended that no positive HIV result be given until after a repeatedly reactive antibody EIA was confirmed by a positive Western blot.[13] Thus, a specimen was obtained at the initial visit and sent to a laboratory for testing, and a second in-person visit was necessary to obtain test results, both positive and negative. In the late 1990s, second-generation EIAs incorporated synthetic HIV-1 protein and recombinant peptide antigens instead of whole viral lysate and added specific antigens to detect HIV-2. These modifications improved the sensitivity and specificity of the assays, but also added HIV-2 tests to the recommended confirmatory sequence after negative or indeterminate Western blot results.[14] Second-generation EIAs still detected only IgG antibody, but shortened the antibody-negative window period by about 9 days compared with first-generation tests.[15]

Tables 1 and **2** list immunoassays approved by the Food and Drug Administration (FDA) for HIV diagnosis as of May 2019. Profound changes in testing technology have revolutionized laboratory testing for HIV, starting with the introduction of rapid HIV antibody assays beginning in 2002[16] and their subsequent eligibility for waived status under the Clinical Laboratory Improvement Amendments of 1988 (CLIA).[17] CLIA-waived tests can be used in outreach settings and other venues by persons with no previous laboratory training, but only with unprocessed specimens, venous or finger-stick whole blood or oral fluid. (When the same tests are used with serum or plasma, they are classified as moderate complexity and are subject to personnel requirements and more regulatory oversight.) Point-of-care rapid tests dramatically improved receipt of test results by obviating a return visit to obtain them.[18]

RAPID HUMAN IMMUNODEFICIENCY VIRUS ANTIBODY TESTS

At the time rapid HIV tests were introduced 10 to 15 years ago, their performance was equivalent to or better than that of the second-generation conventional HIV assays in widespread use.[19] Most single-use rapid HIV tests are based on second-generation principles, using HIV antigens embedded either on a lateral flow strip

Table 1
Food and Drug Administration–approved rapid and point-of-care human immunodeficiency virus tests

Test[a]	Specimen Types	Antigenic Markers Used for Detection	Principle	Generation
OraQuick Advance Rapid HIV-1/2 Antibody Test	Oral fluid, whole blood; plasma	gp41, gp36	Lateral flow	Second
Reveal G4 Rapid HIV-1 Antibody Test	Whole blood, serum, plasma	gp41, gp120	Flow through	Second
Uni-Gold Recombigen HIV-1/2	Whole blood; serum, plasma	gp41, gp120, gp36	Lateral flow	Third
Multispot HIV-1/HIV-2 Rapid Test	Serum, plasma	gp41, gp36	Flow through	Second
Chembio HIV 1/2 Stat Pak Assay	Whole blood; serum, plasma	gp41, gp120, gp36	Lateral flow	Second
Chembio Sure Check HIV 1/2 Assay	Whole blood; serum, plasma	gp41, gp120, gp36	Lateral flow	Second
INSTI HIV-1 Antibody Test Kit	Whole blood; serum, plasma	gp41, gp36	Flow through	Second
Chembio DPP HIV 1/2 Assay	Oral fluid, whole blood, serum, plasma	gp41, gp120, gp36	Dual path platform	Second
Alere Determine HIV 1/2 Ag/Ab Combo	Whole blood, serum, plasma	gp41, gp120, gp36; p24 antibodies	Lateral flow	Fourth
Geenius HIV 1/2 Supplemental Assay	Whole blood, serum, plasma	p24, p31, gp41, gp160, gp36, gp140	Dual path platform	Second

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

(immunochromatography) or in a flow-through membrane (immunoconcentration) to capture antibodies. Antibody detection is accomplished by colloidal gold conjugated to protein A, which binds with high affinity to IgG antibodies[20] and produces a visible colored line or spot when antibodies bind to the HIV antigens or anti-IgG control.[21] Experiments with panels of specimens collected before and during seroconversion demonstrate lateral flow rapid antibody tests become reactive a few days before or at the same time as the HIV-1 Western blot.[22,23] Flow-through rapid assays detect antibodies several days sooner.[24] The dual path platform is another rapid test technique in which specimen is added to a sample pathway that flows toward the long edge of a test strip, onto which one or more antigens have been applied. Buffer is then added that flows across the test strip in a perpendicular direction, activating the detection reagent (colloidal gold conjugated to protein A) that then binds to anti-HIV antibodies, if present. The dual path principle appears to be especially useful for multiplex testing for different antibodies on the same strip, as used by the FDA-approved HIV-1/2 differentiation assay.

THIRD-GENERATION ANTIBODY ASSAYS

An EIA based on novel technology received FDA approval in 1992. This third-generation EIA also used synthetic and recombinant antigens to bind anti-HIV

Table 2
Food and Drug Administration–approved instrumented human immunodeficiency virus immunoassays

Test	Antigenic Markers Used for Detection	Analytes Detected	Generation
EIAs			
Avioq HIV-1 Microelisa System	Viral lysate, gp160	IgG antibodies	Second
Bio-Rad GS HIV-1/2 *PLUS* O	Recombinant p24, gp160, HIV-2 gp36, synthetic group O peptide	IgG & 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 & IgM antibodies p24 antigen	Fourth
CIAs			
Abbott Architect HIV Ag/Ab Combo	Synthetic and recombinant gp41 and HIV-2 gp36, group O peptide, anti-p24 monoclonal antibodies	IgG & IgM antibodies P24 antigen	Fourth
Ortho Vitros Anti-HIV 1 + 2	Recombinant p24, gp41, gp41/120, HIV-2 gp36	IgG & IgM antibodies	Third
Siemens Advia Centaur HIV 1/O/2	Recombinant gp41/120, p24, HIV-2 gp36, group O peptide	IgG & IgM antibodies	Third
Siemens Advia Centaur HIV Ag/Ab combo	Recombinant gp41/120, HIV-2 gp36, group O peptide, anti-p24 monoclonal antibodies	IgG & IgM antibodies p24 antigen	Fourth
Roche Elecsys Combi PT	Recombinant gp41, HIV-2 gp36, HIV-1 RT, HIV-2 RT	IgG & IgM antibodies p24 antigen	Fourth
Ortho Vitros Combi	Recombinant gp41/120, HIV-2 gp36, group O peptide, anti-p24 monoclonal antibodies		Fourth
Multiplex flow immunoassay			
BioRad Bioplex 2200 HIV Ag-Ab	Recombinant gp160, HIV-2 gp36, group O peptide, anti-p24 monoclonal antibodies	IgG & IgM antibodies p24 antigen (detection and differentiation)	Fourth

antibodies, but for detection, used enzymes conjugated to HIV antigens instead of anti-IgG. This "sandwich" principle (antibodies sandwiched between 2 antigens) allowed binding to and detection of both IgM and IgG antibodies and shortened the time from HIV acquisition to detection to 20 to 25 days. The third-generation assay was adopted for screening blood donors but did not come into routine clinical use until the remaining first-generation test was withdrawn from the market in 2007.[25] At about that same time, manufacturers began to replace EIAs with chemiluminescent immunoassays (CIAs), also based on third-generation sandwich principles, that use particles coated with HIV antigen to capture HIV antibodies.[26–28] HIV antigen conjugated to a

luminescent chemical adheres to both IgG and IgM anti-HIV antibodies, and reaction with the luminescent marker emits light, measured as relative light units. Advantages of CIAs include shorter incubation and reaction times than EIAs, which can reduce testing time to as little as 30 to 60 minutes, and their suitability for random access analyzers that can run specimens either one at a time or in batches and thus deliver rapid results from instruments designed for automation and high throughput. Third-generation EIAs and CIAs incorporate specific antigens to detect antibodies against HIV-1, HIV-2, and HIV-1 group O (see **Table 2**).

FOURTH-GENERATION ANTIGEN/ANTIBODY COMBINATION ASSAYS

Fourth-generation EIAs and CIAs, termed antigen/antibody (Ag/Ab) combination assays, add monoclonal anti-p24 antibodies to the recombinant viral antigens to simultaneously detect viral p24 antigen in addition to HIV-1 and HIV-2 antibodies. p24 antigen can be detected directly because each HIV-1 virion contains approximately 2000 to 3000 copies of the p24 molecule, compared with 2 RNA molecules. Detection thresholds for p24 antigen during acute infection correspond to approximately 30,000 viral RNA copies per milliliter.[29] p24 antigen becomes detectable by HIV Ag/Ab combination assays about 5 days after plasma viral RNA,[30] but only transiently. Once anti-p24 antibodies develop, they form immune complexes with p24 antigen and block its detection. Most Ag/Ab combination assays produce a binary positive/negative result and do not identify which component (antigen or antibody) caused the reactivity, with 2 exceptions. The Alere Determine HIV-1/2 Combo Ag/Ab (Abbott Diagnostics, Waltham, MA, USA) is a CLIA-waived lateral-flow rapid test that uses the antigen sandwich technique with a colloidal selenium conjugate to detect IgG and IgM antibodies at 1 location on the test strip and p24 antigen at a separate location.[31] In 2015, the FDA approved the Bio-Plex 2200 (Bio-Rad Laboratories, Hercules, CA, USA), an instrument based on a bead multiplexing technique that 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 the test to differentiate which component (HIV-1 antibody, HIV-2 antibody, or p24 antigen) caused reactivity.[32]

DURATION OF THE WINDOW PERIOD

Each newer generation of immunoassays improved sensitivity for early HIV-1 infection and narrowed the interval between the time of HIV acquisition and its detection (see **Fig. 3**). A recent analysis used modeling to estimate the eclipse period between infection and first detection of RNA (median duration 11.5 days, 99th percentile 33 days) and combined these estimates with test results from plasma seroconversion panels to estimate the median window period and the 99th percentile for each class of immunoassays (**Table 3**).[33] (These window periods are estimates for testing plasma specimens. Testing finger-stick whole blood or oral fluid adds an additional delay in time to detection of 1–3 weeks.[34–36]) The 99th percentile estimate helps to select an appropriate interval for retesting after a possible exposure to be certain infection has not occurred. The CDC recommends retesting serum or plasma at least 45 days after a negative Ag/Ab combination test or after at least 90 days for all other HIV tests.[37]

CURRENT RECOMMENDATIONS FOR LABORATORY TESTING AND RECENT UPDATES

HIV-1 Western blot had long been the gold standard for confirmation after a reactive initial immunoassay,[13] but its shortcomings became increasingly apparent. Numerous

Table 3
Window periods between human immunodeficiency virus acquisition and detection by different test technologies

Type of Test	Median Window Period, d	99th Percentile, d
Instrumented fourth-generation laboratory assay	17.8	44.3
Instrumented third-generation laboratory assay	23.1	49.5
Single-use rapid antibody test	31.1	56.7
HIV-1/HIV-2 differentiation assay	33.4	58.2
Western blot	36.5	64.8

Data from Delaney KP, Hanson DL, Masciotra S, et al. Time Until Emergence of HIV Test Reactivity Following Infection With HIV-1: Implications for Interpreting Test Results and Retesting After Exposure. Clin Infect Dis. 2017;64:53–59.

studies documented high levels of HIV-1 RNA in persons who were reactive by sensitive immunoassays but negative or indeterminate by Western blot.[38,39] In addition, because of cross-reactivity, the 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. The 2014 updated CDC testing algorithm sought to improve diagnosis of early infections, reduce indeterminate results, accurately diagnose HIV-2 infection, and determine its prevalence. The recommended testing sequence, updated as of January 2018, is shown in **Fig. 4**: initial testing with an Ag/Ab combination assay, reflexing reactive specimens to a second-generation HIV-1/HIV-2 antibody differentiation assay.[8] Reactive results on these two tests identify most HIV infections (those that are positive for HIV-1 IgG antibodies) and reduces turnaround time for confirmation compared with Western blot because the FDA-approved HIV-1/HIV-2 differentiation assay is a rapid test. The key feature of the updated algorithm is the third step: HIV-1 RNA testing for those specimens negative

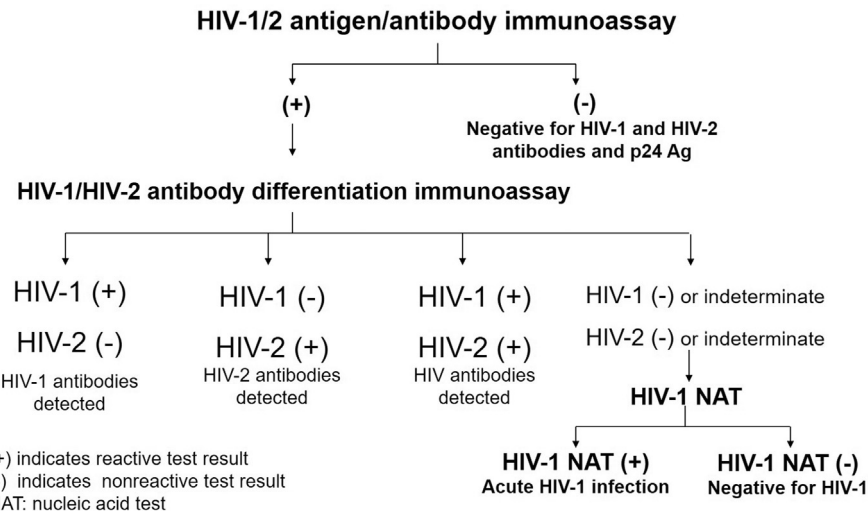

Fig. 4. Recommended laboratory testing algorithm for serum or plasma specimens. Updated January 2018. (*From* 2018 Quick reference guide: recommended laboratory HIV testing algorithm for serum or plasma specimens. Available at: https://stacks.cdc.gov/view/cdc/50872.)

for anti-HIV IgG antibodies. However, only 1 differentiation assay, the Multispot HIV-1/HIV-2 rapid test (Bio-Rad Laboratories), and 1 HIV-1 RNA assay, the APTIMA HIV-1 RNA Qualitative assay (Hologic Inc, San Diego, CA, USA), were FDA approved for diagnosis.

In 2016, Multispot was withdrawn from the market and replaced by the Geenius HIV-1/2 supplemental assay (Bio-Rad Laboratories, Redmond, WA, USA). The Geenius test strip incorporates 4 separate HIV-1 antigens (p24, p31, gp41, and gp160) and 2 HIV-2 antigens (gp36 and gp140) to differentiate HIV-1 from HIV-2 antibodies. The rapid test cartridge uses an automated reader and software that provides 8 possible interpretations according to a proprietary algorithm based on the presence and intensity of the bands (**Table 4**). These interpretations include 2 results not generated before: HIV-2 indeterminate and HIV indeterminate. For either of these test results, CDC recommends repeating the test, and if still reactive, conducting an HIV-1 RNA test to exclude acute HIV-1 infection. If the HIV-1 RNA is negative, supplemental HIV-2 testing (antibody or RNA testing, not FDA approved but available from some commercial laboratories, public health laboratories, and CDC) should be performed, or the testing sequence should be repeated in 2 to 4 weeks.[40]

Since CDC issued the 2014 recommendations, evidence accumulated to show that, with serum or plasma, the Determine Combo rapid test became reactive earlier in seroconversion than instrumented antibody-only third-generation tests, but later than instrumented fourth-generation assays. In a 2017 technical update, CDC reiterated that instrumented Ag/Ab combination tests are preferred for initial testing because of their superior sensitivity, but for laboratories in which instrumented Ag/Ab testing is not feasible, the Determine HIV-1/2 Combo Ag/Ab rapid test can be used with serum or plasma as the first step in the testing algorithm.[41] In contrast, evidence suggests that when used with whole blood, the Determine Combo rapid test showed a significant delay in reactivity compared with plasma and rarely detects p24 antigen during acute infection.[34,42] With finger-stick whole blood, its sensitivity during early infection is similar to that of flow-through rapid HIV antibody tests.

Table 4
Interpretations of the results of the Geenius human immunodeficiency virus-1/2 supplemental assay

HIV-1 Result	HIV-2 Result	Assay Interpretation
Negative	Negative	HIV negative
Indeterminate	Negative	HIV-1 indeterminate[a]
Negative	Indeterminate	HIV-2 indeterminate[b]
Indeterminate	Indeterminate	HIV indeterminate[c]
Positive	Negative	HIV-1 positive
Positive	Indeterminate	HIV-1 positive
Negative	Positive	HIV-2 positive
Indeterminate	Positive	HIV-2 positive
Positive	Positive	HIV-2 positive with HIV-1 cross-reactivity
Positive	Positive	HIV positive untypeable (undifferentiated)

[a] HIV-1 bands detected but did not meet the criteria for HIV-1 positive.
[b] HIV-2 bands detected but did not meet the criteria for HIV-2 positive.
[c] HIV bands detected but did not meet the criteria for HIV-1 positive or HIV-2 positive.

CHALLENGES AND OPPORTUNITIES FOR IMPROVEMENT

Testing with Ag/Ab combination assays has succeeded in identifying acute HIV infections in routine HIV screening programs,[43] and in high-risk populations, they identified 82% of the antibody-negative infections otherwise detectable only by RNA.[44] False-positive results are rare: fewer than 2 per 10,000 test with assays currently in use. This high specificity calls into question the diagnostic value added by a second, corroborating HIV-1 antibody test, and with few reports of HIV-2 infections, it no longer appears warranted to test all fourth-generation reactive specimens for HIV-2 antibodies.[45]

With current technology, it would be more efficient to reverse the current confirmatory testing sequence and, after a reactive Ag/Ab combination assay, perform a quantitative HIV-1 RNA viral load test to both confirm the diagnosis and contribute to immediate clinical management. Based on the specificity of fourth-generation tests, a viral load would be clinically recommended as a next step for the 99.6% of those with a reactive result who are HIV-1 positive, with either a new or previous diagnosis. The US Department of Health and Human Services Panel on Antiretroviral Therapy Guidelines recommends initiation of ART for all persons with HIV-1 infection immediately upon diagnosis to reduce the risk of disease progression and to prevent HIV transmission.[46] Treatment of HIV infection in the earliest stages of acute infection preserves gut-associated immune responses and limits seeding of the long-lived viral reservoir, suggesting that very early ART may help reduce inflammation and HIV-related comorbidities over the long term.[47,48] Early treatment also quickly and substantially reduces transmission.[49] ART is therefore considered urgent in acute HIV, and optimally, initiated on the same day of diagnosis, before HIV resistance genotyping results.[50] Performing an immediate viral load test without an intervening antibody test could facilitate earlier initiation of ART and other aspects of HIV care.

Six HIV-1 RNA viral load tests are available for clinical laboratories with sensitive limits of detection that can establish the presence of HIV-1 RNA (**Table 5**), but so far they are FDA approved only to assess prognosis and monitor response to ART.

Table 5
Food and Drug Administration –approved quantitative viral load assays and specimen requirements

Test and Manufacturer	Amplification Method; Target	Range (Copies/mL)
Amplicor HIV-1 Monitor version 1.5 (Roche Diagnostics, Indianapolis, IN, USA)	RT-PCR; *gag* gene	
Standard		400–750,000
Ultrasensitive		50–100,000
Cobas AmpliPrep/Cobas TaqMan HIV-1 Version 2.0 (Roche Diagnostics)	Real-time RT-PCR; *LTR, gag* gene	20–10,000,000
RealTime HIV-1 (Abbott Molecular, Des Plaines, IL, USA)	Real-time RT-PCR; integrase gene	40–10,000,000
Versant HIV-1 RNA 3.0 (bDNA) (Siemens Healthcare Diagnostics, Tarrytown, NY, USA)	bDNA; *pol* gene	75–500,000
NucliSens HIV-1 QT (BioMérieux, Inc, Durham, NC, USA)	NASBA; *gag* gene	176–3,470,000
APTIMA HIV-1 Quant (Hologic Inc)	TMA; LTR, *pol* gene	30–10,000,000

The qualitative HIV-1 RNA assay, available at far fewer laboratories, is FDA approved for diagnosis,[51] but confirming with the qualitative test adds expense with little additional clinical utility. Without a diagnostic indication, laboratories cannot reflexively conduct a viral load test after a reactive Ag/Ab combination assay, but clinicians can order one until such time that a manufacturer obtains a diagnostic indication, as recently happened with the hepatitis C viral load test.[52]

Current HIV-1 RNA assays are expensive, time-consuming, and require a sophisticated laboratory, but technologic advancements may expand opportunities to use HIV-1 RNA assays for HIV diagnosis. Two simplified rapid HIV-1 RNA tests with short (1–2 hour) turnaround times are now commercially available outside the United States: one is qualitative or semiquantitative[53] and the other is a quantitative viral load assay.[54] Both are suitable for near-patient diagnosis and could add timely and actionable information during the same clinic visit. Neither has yet been submitted for FDA approval.

An undetectable HIV-1 RNA result is not sufficient to rule out HIV infection after a reactive fourth-generation assay. HIV-1 RNA was undetectable in 2% to 4% of specimens that were immunoassay reactive and positive on HIV-1 Western blot.[22,39,55] Some percentage of these might have been from persons on ART, but to achieve final resolution of the testing algorithm, specimens negative for HIV-1 RNA should be tested with the HIV-1/HIV-2 antibody differentiation assay to identify any specimens positive for HIV-1 antibodies or the occasional HIV-2 infection.

THE NEXT FRONTIER: THE EFFECT OF EARLY TREATMENT AND PREEXPOSURE PROPHYLAXIS ON DIAGNOSTIC TEST RESULTS

The new emphasis on rapid, even same-day treatment initiation, especially during acute infection, promises to pose challenges for HIV diagnostics.[56] Early initiation of ART profoundly alters the evolution of HIV biomarkers and the reactivity of immunoassays as depicted in **Fig. 5**, compared with the more predictable sequence in **Fig. 3**.[57] The performance of second-, third-, and fourth-generation screening immunoassays and Western blots were recently rigorously characterized in a large cohort of participants who initiated ART during acute HIV infection.[58] Suppressing viremia during early phases of infection altered the maturation of antibody responses against different HIV-specific antibodies, especially when treatment was started when RNA but not p24 antigen was detectable. Antibody seroconversion did not fully evolve, and seroreversion occurred due to viral suppression. Among 96 participants who were started on ART before any antibody was detectable, 46%, 7%, and 29% were nonreactive on second-generation, third-generation, and fourth-generation immunoassays, respectively, after 26 weeks of treatment. The lower frequency of nonreactive results obtained with the third-generation immunoassays was attributed to the greater breadth of antigens (gp41, p24, gp160) versus gp41 only in the fourth-generation immunoassay, as is the case with FDA-approved third- and fourth-generation assays. Although this study did not report changes in the concentrations of antibodies to specific antigens, 1 study in HIV-infected subjects with a rapid test that detects gp41 antibody only reported gp41 reversion and false-negative results in participants initiating ART early after HIV diagnosis.[59]

These findings likely have ramifications for persons who might become infected with HIV while taking antiretrovirals for preexposure prophylaxis (PrEP). During clinical trials, persons who became infected while taking PrEP demonstrated attenuated seroconversion, with elongation of the intervals between the appearance of each of the laboratory markers and longer delays before infection was detected by rapid tests than persons who became infected while taking placebo.[60] In another trial,

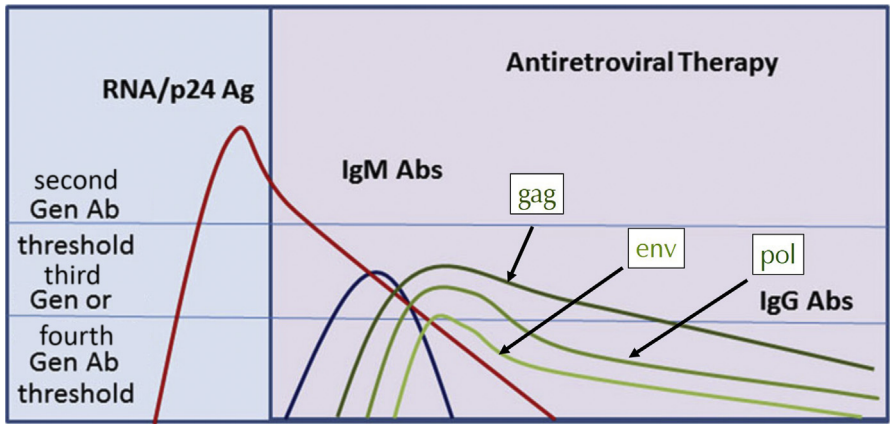

Fig. 5. Effect of ART during acute HIV infection on laboratory markers of HIV-1 infection. After HIV infection, p24 antigen (Ag; *red line*), IgM (*blue line*), and IgG (*green lines*) antibody (Ab) seroconversion occur, which are progressively detected by fourth-, third-, or second-generation assays over the weeks after infection. Diverse Ag-specific IgG responses can be differentially detected during this period by Western blot and other confirmatory assays (indicated by gag, pol, and env *green lines*). Sustained antigenic stimulation is required for maturation and maintenance of these Ab responses. Early treatment with ART aborts the development of antibodies if treatment is initiated very early, and subsequent seroreversion may occur if treatment is initiated shortly following seroconversion, making it difficult to detect or confirm HIV infection by standard diagnostic tests. (*From* Keating SM, Pilcher CD, Busch MP. Editorial commentary: timing is everything: shortcomings of current HIV diagnostics in the early treatment era. Clin Infect Dis 2016;63:562–4; with permission.)

participants who became infected while receiving tenofovir took longer to develop a reactive oral fluid rapid test result than those receiving placebo, suggesting a blunted antibody response.[61]

Four cases of indeterminate or ambiguous HIV test results from recommended testing algorithms have been reported recently in persons who acquired HIV infection while adherent to daily doses of PrEP.[62] These patients tested reactive with Ag/Ab combination assays, but supplemental tests, including HIV-1/2 differentiation assays, Western blot, and HIV RNA tests, when done, were inconsistently positive. In 1 case, findings included a reactive Ag/Ab combination assay, persistently negative Multispot HIV-1/2 supplemental tests, and detectable but not quantifiable HIV-1 RNA, consistent with low-level HIV-1 viremia and immune system preservation without seroconversion.[63] A third drug was added to the PrEP regimen, and the ambiguous test results persisted. RNA viral load remained extremely low, but coding regions were eventually amplified from HIV-1 DNA extracted from isolated $CD4^+$ lymphocytes. In a second case, the Ag/Ab combination assay was reactive, and only gp160 antibodies were present on Western blot. Plasma RNA was undetectable, and neither HIV RNA nor DNA could be isolated from mononuclear cells. PrEP was suspended, and HIV-1 RNA became detectable 17 days later, after which the full antibody response evolved on the Western blot. Although breakthrough infections on PrEP are expected to be few, those that do occur might present dilemmas for diagnosis. Repeated false-positive Ag/Ab combination assay results are rare but especially problematic in the context of PrEP.[64]

If recommended tests produce ambiguous results, repeat testing a few days to weeks later may resolve them if the ambiguities were due to very early infection or

technical issues, such as a mislabeled specimen. If the ambiguous results remain unresolved, it might be useful first to repeat testing with an assay from a different manufacturer or with a different antigen composition. Concordant reactive results on 2 different antibody assays are strongly suggestive of infection. Further investigation if results remain ambiguous would include testing plasma and $CD4^+$ cells for total HIV-1 nucleic acid and proviral DNA, but these are research use assays available only at select laboratories.[62]

SUMMARY

HIV diagnostics have evolved in tandem with a changing social context and advances in therapeutics since 1985, when the first EIA for anti-HIV antibodies was approved for screening blood donations. In that same year, the Public Health Service funded alternative test sites where persons who wanted an HIV test could obtain one without donating blood to do so,[65] making available anonymous HIV testing on demand and effectively removing much of HIV testing from health care settings. Before effective therapy and with potential for substantial stigma, recommendations for diagnostic testing emphasized specificity, requiring confirmation of a highly accurate antibody test with a second, more specific antibody test. Once effective therapy became available, the benefits from an early and accurate HIV diagnosis became increasingly apparent. Rapid tests that detect IgG anti-HIV antibodies in finger-stick blood and oral fluid specimens were introduced, allowing HIV testing to expand to even more nonclinical settings, increasing the number of people aware of their HIV status and allaying many fears about adverse consequences from preliminary false-positive results.

Rapid test technology has seen minimal change since the tests were introduced, but instrumented laboratory assays have evolved considerably in the last 10 years. New immunoassay techniques that detect both IgM and IgG antibodies identify HIV infections sooner, and the ability to directly detect viral p24 antigen allows routine serologic screening to detect HIV-1 infection at just about 2 weeks after acquisition, 5 to 6 days after RNA first appears. Current testing recommendations specify an initial Ag/Ab combination assay, followed, if reactive, by an HIV-1/HIV-2 antibody differentiation assay. If those antibody test results are negative or indeterminate, an HIV-1 RNA assay is performed to identify acute HIV infections.

The new emphasis on rapid treatment initiation suggests performing an HIV-1 RNA viral load assay as the next step after a reactive Ag/Ab combination assay might confirm infection and facilitate earlier ART initiation. The HIV-1/HIV-2 differentiation assay would be reserved for specimens that were negative for HIV-1 RNA. Earlier initiation of ART and administration of PrEP are likely to complicate HIV diagnostics because lack of antigenic stimulation alters the evolution of biomarkers, affecting the reactivity of immunoassays. Technologic advancements promise to expand opportunities to use rapid, easy-to-use HIV-1 RNA assays that can help foster the transition from diagnostic testing to treatment for persons with either newly identified or previously diagnosed HIV infection.

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