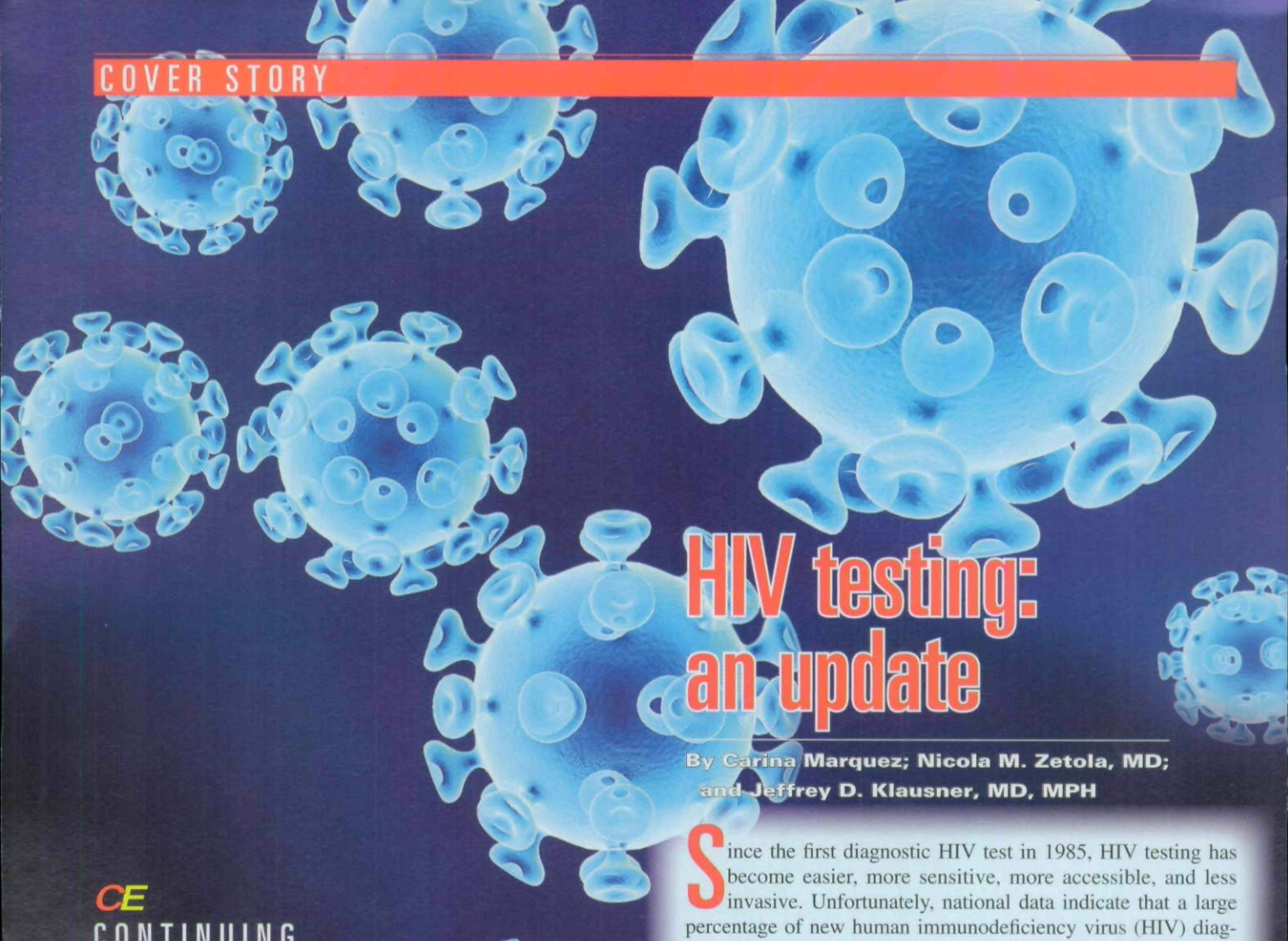

HIV testing: an update

By Carina Marquez; Nicola M. Zetola, MD; and Jeffrey D. Klausner, MD, MPH

Since the first diagnostic HIV test in 1985, HIV testing has become easier, more sensitive, more accessible, and less invasive. Unfortunately, national data indicate that a large percentage of new human immunodeficiency virus (HIV) diagnoses are made in the late stages of disease. In the United States, 40% of patients with newly diagnosed HIV infection develop AIDS within one year of testing.[1] Furthermore, it has been estimated that one-fourth of the people living with HIV infection in the United States do not know they are infected and, thus, miss the opportunity to receive life-saving antiretroviral therapy.[1] That lack of awareness of infection is critical from the public-health perspective as it is estimated that over 50% of new sexually transmitted HIV infections are due to people unaware of their HIV infection; and evidence strongly suggests that once individuals are made aware of their infection, they reduce their risk behavior and decrease the probability of transmitting infection.[2] Therefore, HIV testing provides an opportunity for decreasing the continued incidence of HIV infection and for providing life-saving therapy to newly diagnosed patients.

To decrease the number of people unaware of their HIV infection, in 2006, the Centers for Disease Control and Prevention (CDC) expanded its HIV-testing recommendations.[3] The new CDC recommendations advocate voluntary "opt-out" HIV screening in healthcare settings for all adults instead of just screening traditionally "high-risk" patients. The recommendations also suggest eliminating requirements for written consent for HIV testing, annual re-testing for persons with known risk factors, and third-trimester screening for women who test negative for HIV early in pregnancy. This article will review the current HIV-testing types, with a focus on the role of the various testing modalities in both the public-health and clinical arenas. An additional emphasis will be placed on newer testing modalities and strategies, specifically rapid testing and nucleic-acid amplification testing (NAAT).

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LEARNING OBJECTIVES

Upon completion of this article, the reader will be able to:

1. List the new CDC recommendations for HIV testing.
2. Describe the virology of HIV.
3. List and explain the methodology of the different screening assays used to detect HIV.
4. List common symptoms of patients who have an acute infection with HIV.
5. Define NAAT and explain when it is used.
6. Identify and explain the methodology of rapid HIV antibody tests.
7. List the advantages and disadvantages of urine HIV testing.
8. Discuss the benefits and the problems of at-home HIV testing.
9. Explain what is meant by an indeterminate result on HIV confirmatory assays.

HIV virology and natural history

Our increasing understanding of HIV-transmission kinetics and the clinical course of the infection has changed the strategies used for HIV testing. HIV is a retrovirus that consists of an envelope and a viral core. The viral envelope is taken from the membrane of a human cell during viral budding and carries Env — a complex viral protein. Env consists of a cap (made from glycoprotein gp120) and a stem (made of gp41). Within the envelope, the viral capsid is made of thousands of copies of another viral protein, p24. These three proteins are highly antigenic and are used in many diagnostic tests. The capsid surrounds two single strands of HIV RNA, each of which has a copy of the virus's nine genes.

The new CDC recommendations advocate voluntary "opt-out" HIV screening in healthcare settings for all adults instead of just screening traditionally "high-risk" patients.

HIV is among the most genetically variable of human pathogens. In the United States, HIV-1 accounts for the great majority of cases. A similar but different virus, HIV-2, is a common cause of AIDS in several areas of western Africa but is rare in the United States. In 1998, the CDC reported only 79 cases of HIV-2 infection in the United States.[4] A great variety of HIV-1 strains have been identified and further divided into groups. HIV-1 group M strains are responsible for the most HIV infections worldwide, but the global distribution of HIV is complex and dynamic, and the prevalence of different strains could change rapidly. HIV-1 group O is endemic in Cameroon; and while it is uncommon in America, a case of HIV-1 group O was first reported in the United States in 1996.[5]

Host and viral markers of infection are used for diagnosis and can change during the natural history of HIV infection.[6] Within the first few days of HIV infection, the virus starts an active replication, enabling the detection of viral RNA. Viral antigens (proteins) become detectable soon after. The protein used most widely in HIV diagnosis is the capsid protein p24, which appears usually within the first two to three weeks after acute infection. Its presence in the serum is transient, and its disappearance coincides with the development of both humoral and cellular responses to infection. A transient immunoglobulin M (IgM) antibody response against the capsid or envelope proteins is usually the first to appear and is followed by a long-lasting immunoglobolin G (IgG) response. The appearance of IgG antibodies against the core (p24) and envelope (gp160, 120, 41) proteins are then followed by antibodies against HIV viral enzymes. The development of detectable antibodies against different HIV proteins is called HIV seroconversion and marks the end of what is called the "window period." Acute HIV infection is a term usually referring to the dynamic period between HIV infection and seroconversion.

Acute HIV infection is followed by a period of clinical latency that may last up to 10 years or longer. During this time, viral replication remains active and causes persistent activation of the immune system and subsequent progressive decline in CD4 T-cell lymphocyte counts. Both viral and host factors will determine the rate of decline in the CD4 T-cell count which, ultimately, results in loss of immune function and increased susceptibility to opportunistic infections.

HIV-testing technologies

HIV tests can be characterized as either (1) screening tests or (2) confirmatory tests. The standard testing algorithm is to do a screening test, followed by a confirmatory test for any positive result. Regardless of the type of screening method used, a specimen producing a positive result will be tested again with the same or a different screening test. If that sample is repeatedly reactive, the sample will be assessed with a confirmatory assay. Screening assays, which include the conventional tests (enzyme immunofluorescence assays) and rapid tests, have a high sensitivity for serum antibody and, thus, give few false-negative results. Confirmatory assays include Western Blots (WB), indirect immunofluorescent antibody assays (IFA), and, recently, HIV RNA detection by NAAT. All these tests have a high specificity, meaning that a positive test has a very low probability of being a false positive.

Screening technologies

Enzyme immunoassays (EIA)

The enzyme immunoassay (EIA) is the most common screening test used. It is simple and amenable to high-volume testing, and

Continues on page 14

Generation	Mechanism	Approximate window period	Name	Sample	Target molecule	Manufacturer	FDA cleared
First and second generation	Viral lysate used to bind patient HIV Ab. Detects IgG antibody to HIV viral proteins. Second generations are the same as first generation, but use purified Ag or recombinant virus.	4 to 12 weeks	Vironostika HIV-1 Microelisa	Serum/Plasma/ Blood spot/Oral fluids	Viral Lysate	bioMerieux Inc.	Yes
			Genetic Systems rLAV EIA [HIV1]	Plasma/Blood spot	Viral Lysate and E-coli recombinant antigen	Bio-Rad Laboratories	Yes
Third generation	Same mechanism as first and second generation, but adds IgM detection, which decreases the window period	3 to 4 weeks	HIVAB HIV-1/HIV-2 (rDNA)	Serum/Plasma	Recombinant HIV-1 env and gag HIV-2 env proteins	Abbott Laboratories	Yes
			Genetic Systems HIV-1/HIV-2 PLUS O EIA	Serum/Plasma	Purified gp160, p24, and peptides representing regions of gp41 from HIV-1 group O and gp36 from HIV-2	Bio-Rad Laboratories	Yes
Fourth generation	Same mechanism as third generation, but in addition uses a antibody to detect p24 antigen in the patients serum	2 weeks	VIDAS HIV DUO Ultra	Serum/Plasma	HIV-1 gp160, p24 antigen, and peptides representing regions of gp41 from HIV-1 group O and gp36 from HIV-2	bioMerieux Inc.	No

Table 1. Selected enzyme immunoassays

has high sensitivity and specificity. Many assays are FDA cleared for use in serum, plasma, finger-stick whole blood, oral fluid, and urine. EIAs use an HIV antigen coated on a microwell plate to detect any HIV antibodies present in a specimen. Four generations of EIAs have been produced (see Table 1) with later generations having improved test performance, and shorter window periods during which antibodies cannot be detected. First- and second-generation tests have a window period of about six to 12 weeks for most individuals.[7] Both first- and second-generation tests detect IgG antibodies to HIV. The first-generation test uses viral lysate as a target antigen, while the second-generation tests use recombinant proteins representing HIV capsid and envelope. Currently, second-generation EIAs are the most frequently used HIV-screening tests in the United States.

HIV-1 group O is endemic in Cameroon; and while it is uncommon in America, a case of HIV-1 group O was first reported in the United States in 1996.

Third-generation tests have the ability to detect IgM antibodies in addition to IgG, through the "antigen sandwich method" in which HIV antibodies from the specimen are sandwiched between two antigen molecules, one in the solid phase and one conjugated to an enzyme, such as alkaline phosphatase or horseradish peroxidase. In addition, third-generation tests have the capacity to detect certain HIV subtypes, particularly HIV-1 Group 0 and HIV-2 which were not included in previous generation tests.

Fourth-generation tests detect IgM and IgG antibodies as well as the presence of the viral capsid antigen p24. The detection of p24 antigen reduces the window period to two weeks, and makes detection of acute HIV infection (that is, HIV infection before seroconversion) possible.[8,9] Currently, there are eight commercial fourth-generation tests, none of which have been FDA cleared. Fourth-generation tests combine two methodologies into one assay — antigen and antibody detection. Recent studies on fourth-generation tests have compared the sensitivity and window period of various assays, with a focus on the most recent fourth-generation test: VIDAS HIV DUO Ultra (bioMerieux, Marcy-l'Etoile, France).[9-11]

Unlike the other fourth-generation tests, the VIDAS HIV DUO Ultra test produces two distinct results for antigen and antibody detection and has the lowest p24 antigen detection limit at 3 pg/mL. Those characteristics allow the differentiation of acute HIV infection from recent and/or established infections. The microwells of the combined antigen-antibody test are designed differently; the upper part of the well is coated with anti-p24 monoclonal antibodies and the lower part of the well is coated with antigens that can detect IgG, IgM, and IgA for HIV-1 and -2. The HIV DUO Ultra test was found to be the most sensitive fourth-generation assay (compared to Enzygnost HIV Integral, Enzymun HIV Combi, Genscreen Plus HIV Ag-Ab, and AxSYM HIV Ag/Ab Combo), and was found to be as sensitive as a single p24 assay (Genetic Systems HIV-1 Ag EIA) — making it a viable option for p24 testing.[10,11]

Screening tests for acute HIV: nucleic-acid amplification testing and fourth-generation EIAs

Acute infections are often missed, as the clinical symptoms associated with acute infection are often absent or subtle, and the standard HIV tests used by clinicians, typically first-generation assays in many developing countries and second-generation tests in the United States, will not identify persons with acute infection. The symptoms, diagnosis, and management of acute HIV infection are beyond the scope of this review and have been recently reviewed elsewhere.[12] Around two weeks into the acute infection, approximately two-thirds of patients have some symptoms attributable to acute retroviral syndrome; the most common symptoms are fever (present in 80% to 90% of patients), malaise, anorexia, myalagias, and headache (in about 50% of patients). Although still controversial, current clinical data suggests that treatment prior to seroconversion may benefit patients in the short term and possibly in the long term by augmenting host immunity and potentially obviating or delaying the need for continuous antiretrovirals.[13,14] Identification of acute HIV infection is also important from a public-health standpoint, as the high levels of viremia, combined with a lack of awareness of infection, make acutely infected individuals a high transmission risk. Mathematical models have suggested that persons with acute HIV infection are the important drivers of the epidemic,[15] and epidemiologic studies suggest persons with recent infection are much more infectious than those who are chronically infected.[16]

In order to diagnose acute HIV infection, it is necessary to detect the presence of HIV antigen in patients that have not yet seroconverted. Therefore, the gold standard for diagnosing acute infection is the use of NAAT in the setting of a negative HIV antibody result.[6] NAAT can be both quantitative and qualitative. Quantitative assays determine the plasma viral load and are used to monitor disease

Continues on page 16

Test name	Specimen type	CLIA category	Sensitivity	Specificity	Detects HIV?
OraQuick	Oral fluid	Waived	99.3% (98.4-99.7)	99.8% (99.6-99.9)	Yes
ADVANCE Rapid	Whole blood	Waived	99.6% (98.5-99.9)	100% (99.7-100)	
HIV 1/2 Antibody Test	Plasma	Moderate complexity	99.6% (98.9-99.8)	99.9% (99.6-99.9)	
Uni-Gold	Whole blood	Waived	100% (99.5=100)	99.7% (99.0-100)	No
Recombigen HIV	Serum and plasma	Moderate complexity	100% (99.5=100)	99.8% (99.3-100)	
Reveal G03 Rapid	Serum	Moderate complexity	99.8% (99.2-100)	99.1% (98.4-99.4)	No
HIV-1 Antibody Test	Plasma	Moderate complexity	99.8% (99.0-100)	98.6% (98.4-98.8)	
MultiSpot HIV-1/HIV-2	Serum	Moderate complexity	100% (99.94-100)	99.93% (99.6-100)	Yes, differentiates
	Plasma	Moderate complexity	100% (99.94-100)	99.91% (99.77-100)	IV-1 from HIV-2
Clearview HIV 1/2	Whole blood	Waived	99.7% (98.9-100)	99.9% (99.6-100)	Yes
STAT-PAK	Serum and plasma	Waived	99.7% (98.9-100)	99.9% (99.6-100)	
Clearview	Whole blood	Submitted for waiver	99.7% (98.9-100)	99.90% (99.6-100)	Yes
COMPLETE HIV 1/2	Serum and plasma	Submitted for waiver	99.7% (98.9-100)	99/9% (99.6-100)	

Table 2. Current rapid HIV tests cleared by the U.S. Food and Drug Administration, 2007

Adapted from Health Research and Education Trust; available at *http://www.hret.org/hret/programs/hivtransmrpd.html* - Updated August 30, 2007

progression and response to antiretroviral therapy. On the other hand, qualitative assays reveal whether HIV RNA is present or not and are used to screen specimens for the presence of HIV antigen. The APTIMA HIV 1 RNA Qualitative Assay (Gen-Probe Inc., San Diego, CA) is the only NAAT cleared by the FDA for (1) diagnosis of primary HIV-1 infection and (2) confirming HIV-1 infection when tests for antibodies to HIV-1 are reactive.

Historically, NAAT has not been included in routine HIV-screening protocols, due to the high cost and time and labor that most of those technologies require. To decrease the costs, blood-donor programs in the United States have been using pooling algorithms with NAAT.[17] In those algorithms, antibody-negative specimens are combined in pools, and each aggregated screening pool is assessed by NAAT. A negative pool ends the screening protocol. If a pool has a positive NAAT result, then the pool is deconstructed further into either smaller intermediate pools or individual specimens until the positive specimen is identified. The primary benefit of specimen pooling is significantly decreased costs compared to testing each individual specimen. The major drawback is that pooling involves the dilution of specimens, which may impact test sensitivity. Recent public-health efforts to diagnosis acute infection have led to the use of pooling with NAAT in routine HIV screening in certain settings.[18-20]

Experiences thus far with the pooling technique for routine testing have been very encouraging, particularly among traditional high-risk populations. Currently, pooled NAAT is being used in public HIV-testing sites in Los Angeles, San Francisco, North Carolina, New York City, Maryland, Washington, DC, Florida, and Seattle-King County. At the public STD clinic in San Francisco, routine screening for acute infection using NAAT resulted in an increase in HIV case detection of 8.8%[21]; and in the state of North Carolina, the addition of NAAT increased the number of cases identified by 3.9%.[19] The study in North Carolina and other studies on the pooled technique in community-screening programs found the protocol cost effective. In North Carolina, the testing cost only increased by $3.63 per specimen.

Currently, sampling-pooling protocols differ in terms of the size of the master pools, the number of intermediate pools, and the type of nucleic-acid amplification test used. Limited data exist on the comparative performance of NAAT tests for the screening of pooled specimens, but tests with lower detection limits are likely to perform better. Further studies are warranted to assess the comparative performance of different NAAT tests for use with pooled samples.[22]

While experiences with NAAT seem promising, the optimal approach to detect HIV infection in conventional settings is still being defined.[23] To date, NAAT has been incorporated into screening protocols primarily in settings that test high-risk individuals. The cost-effectiveness, and positive and negative predictive value in NAAT in low-risk settings is unclear. Third- and fourth-generation EIAs may be an alternative to NAAT in certain settings, as they can identify some acute infections, although less than the number of acute infections identified by NAAT. For example, in Seattle-King County, HIV-RNA screening for acute HIV infections in 2003 revealed seven acute infections via NAAT. Four of the seven samples were available for re-testing, and two of the four specimens were found to be positive with a third-generation EIA.[24] A prospective study in Malawi found that parallel rapid and p24-antigen testing detected approximately 90% of acute HIV cases identified by NAAT.[25] While third- and fourth-generation EIAs are likely to provide an alternative for acute HIV detection in low-income settings, algorithms combining standard EIAs followed by pooled-specimen screening by a NAAT are recommended for settings in which resources are available.

While the benefits of home testing are compelling, there are still many outstanding questions.

Rapid HIV antibody tests

One disadvantage of standard EIAs is that it can take up to two weeks for patients to get results, and many patients in publicly funded testing sites do not return to get their test results. Lack of follow up for test results is a significant problem, and failure to return for test results has been found to occur more frequently among individuals at elevated risk of contracting HIV and individuals who tested positive.[26] Rapid HIV-test results can be received the same day, as testing takes less than 30 minutes to complete, and the test can be done at both clinical and non-traditional sites, such as emergency departments, community centers, and health fairs. In general, rapid tests are preferred by patients in comparison to conventional EIAs, and those tested are more likely to receive their results — especially at non-traditional sites such as needle exchanges or bathhouses.[27,28]

There are four rapid tests cleared for HIV-1/2 detection, one of which, the OraQuick Advance Rapid HIV-1/2 Antibody Test, is cleared for testing of oral fluid (see Table 2). The sensitivity of the tests is comparable to standard second-generation EIA testing.[28] Rapid tests each have a synthetic antigen (the gp41 region of HIV-1, and gp36 for HIV-2) affixed to a test membrane, and a sample (finger-stick blood, venipuncture blood, or oral fluid) is applied to the membrane. If the sample contains antibodies to the gp41 region of HIV, then the membrane will change color. In addition, each test has a goat anti-human IgG antibody for control, and each test requires the periodic use of external controls.

Each rapid test has an assigned Clinical Laboratory Improvement Amendments of 1998 (CLIA'98) category that determines the personnel and the type of facilities required to perform the test. Persons without formal laboratory training and outside the traditional laboratory can perform waived tests. To classify as a CLIA-waived test, the test must use direct, unprocessed specimens (such as oral or whole blood), and must be easy to perform by persons without formal laboratory training. The rapid test is the only HIV-testing modality that can be done outside of the laboratory setting.

Another benefit of the rapid test is that it can be non-invasive, as oral-fluid rapid testing was FDA cleared in 2004. Post-marketing surveillance of the Ora Quick Advance Rapid HIV1/2 Antibody Test on whole blood and oral fluid yielded favorable results.[29] Testing of over 135,000 whole-blood samples and over 26,000 oral-fluid tests yielded a specificity of 99.98% with a positive predictive value of 99.24% for blood, and specificity of 99.89% with a positive predictive value of 90% for oral fluids. While oral fluid was slightly less specific, experts do not discourage its use, as the increased acceptance of the non-invasive method will likely outweigh the small deficit in specificity.[29]

While data on rapid HIV testing are encouraging, their use still requires some caution. Steckler, et al,[30] reported three cases of early infection that were missed by OraQuick Rapid HIV-1

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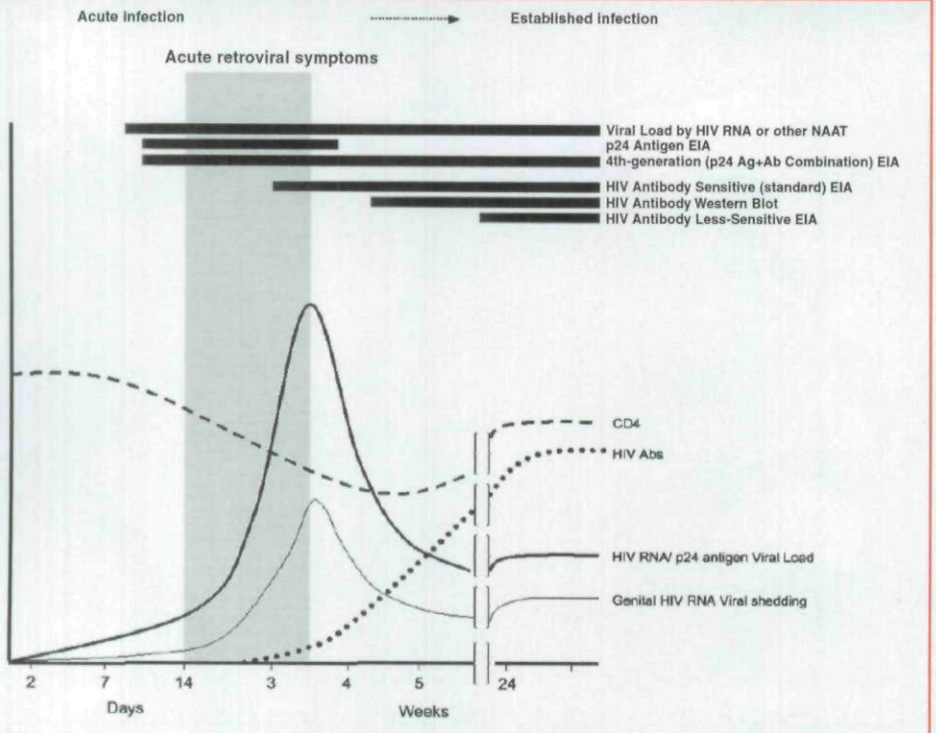

Figure 1. Correlation between HIV diagnostic tests and host and viral markers of HIV infection. The symptomatic period for acture HIV infection is marked in gray. The black bars mark the time when the various diagnostic tests are likely positive. (From Fiebig EW, Wright DJ, Rawal BD, et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. *AIDS*. 2003;17:1871-1879) Ab, antibody; Ag, antigen; EIA, enzyme immunoassay; NAAT, nucleic-acid amplification test.

Antibody Rapid Test, but, subsequently, found to be positive by third-generation EIAs and Western Blots. Although the cases may have been missed due to operator error, those findings support the continued use of RNA pooling within rapid-testing protocols in high-risk populations. Case reports of false-negative test results also emphasize the importance of clinical judgment in the interpretation of negative results.

Still under debate and development: urine tests

Urine HIV tests measure intact HIV IgG antibodies found in urine specimens and have been cleared by the FDA for use with EIA and Western Blot. Assays using urine have the potential to reduce barriers to testing, as they are simple and non-invasive, and the urine can be stored for long periods at room temperature. Despite its advantages, the urine-based HIV test is not commonly used, although test performance may be similar to blood-based testing (in one study the Maxim Urine HIV-1 EIA had a sensitivity of 98.7%).[31]

Rapid urine tests, although commercially available, are not FDA cleared. The Aware-Urine assay (Calypte Biomedical Corp., Rockville, MD), initially had promising preliminary results,[32] but the sensitivity was found to be poor in a recent study set in rural Uganda. The study reported that 942 urine samples yielded a sensitivity of 88.7% and specificity of 99.9% in comparison to EIAs using serum confirmed by Western Blot.[33]

HIV testing at home

Rapid HIV testing at home has the potential to increase access to testing, but its role in public health and testing protocols is still unclear.[34,35] Benefits of home testing are the perception of increased privacy and ease — as the test eliminates the need to attend a publicly funded HIV-testing site or medical provider. The FDA has not yet cleared a complete HIV home-testing kit; however, in 1999, it cleared the first HIV home sample-collection kit — the Home Access HIV-1 Test System. The collection kit allows individuals to mail in a sample of whole blood that was collected at home by finger stick and obtain results by telephone three days later.

The FDA reviewed testimony in 2006 regarding the clearance of a complete rapid HIV home test, the OraQuick ADVANCE 1/2. This test allows individuals to use either fingerstick whole blood or oral fluid as a specimen. The specimen is placed on the test device, and the test device is then placed into a solution vial. Test results are available 20 to 40 minutes after putting the test vial into the developer solution. A purple test line with a positive control line indicates a positive test, whereas a negative test will show only the control line (Oraquick Advance Rapid HIV 1/2 Antibody Test package insert: Orasure Technologies Inc., Bethlehem, PA).

While the benefits of home testing are compelling, there are still many outstanding questions. Current concerns surround the cost; for example, laboratories pay $12 to $17 for each OraQuick kit, and the cost will likely increase for over-the-counter sales. A high cost may make the test unattainable by many high-risk populations and may deter its use by the general public. In addition, reports of unexplained high rates of false-positive OraQuick rapid-test results in a few publicly funded HIV-testing sites raised concerns about the potential for false-positive results in the home setting.[29] Additional questions regarding complete home HIV-testing kits are well summarized by Branson[36]: 1) Do home HIV tests expand access to testing? 2) Can consumers correctly use the home HIV test? 3) Do home HIV-test users experience potential harm because they receive no face-to-face counseling? 4) How do home sample-collection kits affect public-health practices? Given the many unknowns, the FDA has required that the OraQuick manufacturers — at a minimum — demonstrate the device is accurate in the hands of lay users prior to FDA clearance; currently phase II trials are underway.

Confirmatory HIV tests

All positive screening tests must be confirmed by a confirmatory test, either a WB or indirect immunofluorescent antibody assay, and most recently the APTIMA HIV 1 RNA Qualitative Assay was the first nucleicacid amplification test to be cleared by the FDA for confirmatory testing. Confirmatory tests are highly

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specific, more time consuming, and more expensive than most screening assays. IFA is used less frequently as it is expensive and requires highly trained laboratory personnel. The WB is the traditional confirmatory test and is based on the recognition of the major HIV proteins (p24, gp41, and gp120/160), and modified WBs can identify and differentiate between HIV-1 and HIV-2 infections. In WB assays, individual HIV proteins are fractionated by weight via gel electrophoresis and transferred onto nitrocellulose paper. The patient's serum is added to the paper; and if antibodies are present, they will bind to the corresponding antigens. CDC guidelines define a positive result as reactivity to at least two of the major antigens, and a negative result requires the absence of all bands. A reactivity profile that does not meet criteria for either positive or negative results is considered indeterminate.

Indeterminate results may be found in 10% to 20% of EIA positive tests, and the clinical significance should take into account the risk factors and medical status of the patients. Certain indeterminate profiles are more likely to suggest true infection, such as reactivity to p24, p31, and p55, whereas p17 is more likely to be truly negative. Indeterminate results can be a result of non-specific reactions from high levels of non-specific and cross-reactive antibodies. While false-positive test results are very rare with confirmatory assays, they can occur. False positives have been reported in patients with autoimmune disease. Re-testing guidelines for indeterminate results vary and range from two weeks to six months.[7]

Conclusion

In the 25 years since the start of the HIV pandemic, there have been great advances in the virology, immunology, and treatment of HIV infection. Paralleling these advances in virology and clinical medicine has been the development of several different HIV-testing technologies. Since the development of the first HIV test, new technologies have evolved and improved through increasing the sensitivity and specificity of tests — narrowing the window period — thus making it possible to detect HIV infection earlier — and decreasing barriers to case identification by making test results available faster and making non-invasive testing possible. Future avenues of growth would be the improvement of rapid urine tests and the development of rapid RNA testing for oral fluids and finger-stick whole-blood samples. HIV testing, public health, and clinical interventions are inextricably linked; and while current challenges remain, the overall interplay among the three makes improved control and treatment of the disease possible. □

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Sex, human papilloma virus infection, and head and neck cancer

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Over 100 human papillomavirus (HPV) types have been identified, with many linked to cancer. The burden of head and neck cancers is relatively small; it is estimated that 34,360 new cases of head and neck cancer will have been diagnosed in 2007 in the United States, and 7,550 deaths associated with head and neck cancers will occur.[1] Head and neck cancers account for less than 3% of new cancer diagnoses and 1.3% of cancer-related mortality.[1] Although molecular evidence supports the causal role of HPV in squamous-cell carcinomas of the head and neck, epidemiologic data showing an association between HPV and those cancers are lacking. A recent case-control study by D'Souza, et al,[2] adds an epidemiologic perspective to the growing body of scientific literature supporting the role of HPV infection in head and neck cancers.

In D'Souza, et al's, study, characteristics of patients with head-and-neck squamous-cell carcinoma diagnosed in the Johns Hopkins Hospital otolaryngology clinic between 2000 and 2005 were compared with those without a history of cancer seen at the same clinic during the same period. Enrolled patients submitted oral saline rinse, oral mucosal brush, and serum specimens. Researchers used multiplex polymerase chain reaction (PCR) assays targeting the L1 region of HPV to determine the HPV type(s) in tumor specimens, when available. Additionally, they used an enzyme-linked immunosorbent assay (ELISA) to measure serum antibodies to HPV-16 (the HPV subtype most commonly associated with head and neck cancers) L1 protein, and E6 and E7 proteins. The authors use multivariable regression to adjust for age, sex, smoking, alcohol use, dentition, dental-hygiene practices, and family history of head and neck cancers. To elucidate possible pathways in the etiology of head and neck cancers, various statistical interactions among smoking, alcohol use, and HPV infection were explored.

Enrolled cases (n=100) and controls (n=200) were primarily male (86%), less than 65 years old (85%) and white (86%). **The authors found increasing numbers of vaginal and oral sex partners, having had a casual sex partner and never or rarely using condoms, were significantly associated with an increased likelihood of head and neck cancer.** When analysis of sexual behaviors was restricted to only head and neck cancers that harbored HPV-16, those associations were strengthened. Having had a same-gender sex partner or a sex partner with a history of an HPV-related cancer was not associated with head and neck cancer.

Continues on page 22