Frequently Asked Questions: Fourth Generation HIV Ab/Ag Combination Assays

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Background:

Approximately 1.6% of all Philadelphia residents are living with HIV, and the Centers for Disease Control and Prevention (CDC) estimates that one quarter of them are unaware of their status[1]. The CDC recommends that all people ages 13-64 are tested for HIV *regardless of personal risk* to benefit individual and public health. When HIV-infected individuals are diagnosed and linked to care, and their HIV is sufficiently managed soon after acquiring the disease, they experience fewer co-morbidities than individuals who are diagnosed later in the course of infection. HIV-positive individuals who are tested and treated early also experience a life expectancy near that of the non-infected population. Furthermore, individual awareness of HIV status benefits public health by decreasing transmission rates; HIV-infected individuals who are aware of their status often decrease behaviors that lead to HIV transmission. Transmission risk also decreases with reduction of HIV viral levels.

Testing Technology:

Advancements in HIV testing technology and early detection of HIV may maximize the benefits to individual and public health. Among new HIV testing technologies are fourth generation HIV Ab/Ag combination assays. Fourth generation HIV Ab/Ag combination assays differ from previously developed screening technologies in two ways: detection of HIV-1 p24 antigens and quick time to result. The detection of HIV-1 p24 antigens allows for the identification of acute HIV-1 infection. All other HIV screening technologies have window periods exceeding the acute infection period, which may result in false negative tests in the acutely-infected patients. Fourth generation HIV Ab/Ag combination assays may be processed in as few as 29 minutes, expanding the use of conventional HIV screening tests into settings where same-day results are warranted. In contrast, other conventional HIV tests may take days to process, making them difficult to use in many healthcare settings or with certain populations.

Preferred HIV testing algorithms are currently being reconsidered as a result of marked improvements to HIV screening tests over time, notably shrinking "window periods**,**" and increasing sensitivity/ specificity to HIV antibodies. Older testing technologies are becoming obsolete, including use of the Western Blot as the standard HIV confirmatory test. HIV testing algorithms that best utilize advances in testing technology are being considered by a number of organizations, including the CDC, Association of Public Health Laboratories (APHL), Food and Drug Administration, and many professional medical organizations.

This document will address frequently asked questions about fourth generation HIV Ab/Ag combination assays.

1. Brady, K. (2013). HIV Update for Obstetricians: A Look at Local and National Data and Clinical Changes. Philadelphia Department of Public Health. Retrieved from http://www.phila.gov/health//index.html

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Frequently Asked Questions:

How do HIV test generations differ?

HIV antibody tests are categorized by "generation," and higher numbers indicate more recently developed assays. The goals of technological advancement are to shorten the window period, and to improve and maintain high sensitivity and specificity.

First and second generation HIV enzyme immunoassays (EIAs) detect anti-HIV IgG antibodies. Second generation HIV EIAs are currently manufactured for use in healthcare and non-health settings, and they have a window period of 4-6 weeks to detect HIV-1/HIV-2 antibodies. OraQuick, manufactured by OraSure Technologies, Inc., is a commonly used second generation point-of-care HIV test.

Third generation HIV EIAs identify anti-HIV IgM and IgG antibodies for HIV-1/HIV-2. Anti-HIV IgM antibodies can be detected earlier than IgG antibodies, reducing the window period to 3-4 weeks. The Vitros HIV-1/HIV-2 EIA, manufactured by Ortho Clinical Diagnostics, is an example of a conventional third generation HIV test. Uni-gold, manufactured by Trinity Biotech, is a third generation point-of-care HIV test.

Fourth generation HIV Ab/Ag combination assays (fourth generation HIV tests) identify anti-HIV IgM and IgG antibodies for HIV-1/HIV-2, as well as for HIV-1 p24 antigens. HIV p24 levels can be detected 2-4 weeks after HIV exposure, further reducing the window period needed to accurately determine the HIV status of a patient. HIV Ab/Ag combination assays are conventional HIV tests available through Abbott Laboratories and Bio-Rad Laboratories.

What are the benefits of fourth generation HIV Ab/Ag combination assays?

Of all the HIV testing technologies, fourth generation HIV Ab/Ag combination assays are the only tests that are able to identify acute HIV-1 infection due to this generation's ability to detect HIV-1 p24 antigens. Detecting acute HIV-1 infection is significant because positive patients can be diagnosed near the peak of viral replication, when they are most contagious. Approximately half of all new infections originate from HIV-positive people who are unaware of their status. The majority of people diagnosed with HIV reduce behaviors that result in transmission upon learning their HIV-positive status. Furthermore, effective antiretroviral treatment reduces the risk of HIV-positive individuals transmitting the infection, regardless of behavior modification. Diagnosing and engaging patients in HIV care early has the potential to substantially decrease HIV incidence.

Unlike some HIV tests, automated fourth generation HIV tests do not require bracketed controls. Fewer quality assurance checks may result in more efficient processing of HIV tests and results.

What are the limitations of fourth generation HIV Ab/Ag combination assays?
Fourth generation HIV tests detect HIV-1/HIV-2 antibodies, but only HIV-1 p24 antigens. Therefore, acute HIV-2 infection cannot be detected by fourth generation HIV Ab/Ag combination assays. The window period for HIV-2 correlates with detectable antibody levels, similar to that of third generation HIV-2 tests.

Like all HIV testing technologies, false positive results may occur infrequently. Patients with advanced HIV-2 disease may receive false negative results if HIV-2 antibodies are below detectable levels. Variant strains of HIV may also not be detected.

In some settings, processing time may be considered a limitation. While the processing time of fourth generation Ab/Ag combination assays may be faster than other HIV tests, they do not produce results as quickly as rapid HIV testing technologies.

Fourth generation HIV tests do not detect HIV-2 antigens. Is FDA-approved HIV screening technology currently available to detect HIV-2 antigens?
At this time, there are no FDA-approved HIV screening tests with the capacity to detect HIV-2 antigens. Many HIV tests, including fourth generation HIV Ab/Ag combination assays, detect both HIV-1 and HIV-2 antibodies. Patients who are at high-risk of HIV-2 infection should be counseled about the window period of HIV-2 with all testing technologies, which is consistent with antibody production and not viral levels. HIV-2 in the United States is most common among patients originating from West Africa and living in the Northeast[2].

Do positive results from fourth generation HIV Ab/Ag combination assays distinguish between the detection of anti-HIV antibodies and p24 antigens?
No, fourth generation HIV tests provide reactive or non-reactive results. Additional tests are needed to distinguish between HIV-1 and HIV-2, as well as acute and chronic infection. Secondary tests will provide supplementary information to confirm HIV diagnosis.

My patient received a reactive result on a fourth generation HIV Ab/Ag combination assay. What does that mean and what are the next steps?
A reactive result from a fourth generation HIV Ab/Ag combination assay means that HIV-1 p24 antigens, HIV-1 antibodies, and/or HIV-2 antibodies were detected in the sample processed. A secondary test is required to confirm the reactive result. Secondary tests and testing algorithms vary among healthcare facilities. Laboratories and/or healthcare systems may automatically process secondary, confirmatory tests upon the receipt of a reactive HIV test. Check with your healthcare site's administration to determine what processes are required to confirm HIV diagnosis.

2. CDC. HIV-2 Infection Surveillance--- United States, 1987-2009. MMRW 2011 60(29). Retrieved from http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6029a3.htm

What algorithms are approved to diagnose HIV infection in healthcare settings?
The "gold standard" HIV diagnostic algorithm recommended for over two decades includes a preliminary HIV screening test that detects HIV antibodies, followed by a Western Blot or, less commonly, indirect immunofluorescence assay (IFA). Many healthcare settings in the United States use this algorithm.

In 2010 and 2011, several organizations published recommendations to change the "gold standard" HIV diagnostic algorithm due to the shortcomings of Western Blot and IFA tests, including a relatively long "window period" compared to recently developed technologies. CDC and APHL are currently developing guidelines on HIV diagnostic algorithms. They have published a document entitled, "HIV Testing Algorithms: A Status Report," describing effective alternatives to the "gold standard" algorithm.

The report can be accessed on the APHL website:
http://www.aphl.org/aphlprograms/infectious/hiv/Pages/HIV-Diagnostic-Testing-Algorithm.aspx.

Updated guidelines will be published on the CDC website when available:
http://www.cdc.gov/hiv/topics/testing/guideline.htm.

My patient received a non-reactive result on a fourth generation combination HIV Ab/Ag HIV test. What does that mean and what are the next steps?
A non-reactive result from a fourth generation combination HIV Ab/Ag assay means that HIV-1 p24 antigens, HIV-1 antibodies, and HIV-2 antibodies were not detected in the sample processed. No follow-up testing is indicated; however, patients at high risk for HIV-1 acquisition within 2-4 weeks of the test, or HIV-2 within 4-6 weeks of the test, should be re-tested per provider discretion.

Who manufactures fourth generation combination HIV Ab/Ag testing technology?
Abbott Laboratories manufactures the HIV Ab/Ag Combo Assay for their ARCHITECT i1000SR, i2000, and i2000SR platforms. More information can be found on the manufacturer's website:
http://www.abbottdiagnostics.com.au/Products/Instruments_by_Platform/architect.cfm

Bio-Rad Laboratories manufactures the GS HIV Combo Ag/Ab EIA for their EVOLIS Automated Microplate System. The GS HIV Combo Ag/Ab EIA may also be processed manually. Information is provided by the manufacturer: http://www.bio-rad.com/webroot/web/pdf/cdg/literature/P-143.pdf

What is the processing time for fourth generation combination HIV Ab/Ag assays?

The HIV Ab/Ag Combo Assay by Abbott Laboratories can process a single HIV test in 29 minutes and the GS HIV Combo Ag/Ab EIA by Bio-Rad Laboratories can process a single HIV test in 2.5 hours. Minimum time to result varies between tests due to different processing techniques.

Each laboratory will determine time intervals for processing HIV tests. Laboratories may elect to process HIV tests continuously, as needed, or batch tests per pre-determined time interval (i.e. every 8, 12, or 24 hours). The frequency with which tests are processed depends on test volume, cost differential between individual and batch processing, and staffing needs, among other reasons.

What other tests can be processed by the Abbott ARCHITECT and Bio-Rad EVOLIS?

Many tests can be processed on each piece of equipment.

Test menus for Abbott ARCHITECT machines are available from the manufacturer and may be accessed below.

Abbott ARCHITECT i1000SR:
http://www.abbottdiagnostics.com/Products/Instruments_by_Platform/systests.cfm?sys_id=164
Abbott ARCHITECT i2000SR:
http://www.abbottdiagnostics.com/Products/Instruments_by_Platform/systests.cfm?sys_id=79

The test menu for Bio-Rad's EVOLIS Automated Microplate System is available by request from the manufacturer. Email a "quick question" using the link below and a representative will provide you with an EVOLIS system test menu.

Bio-Rad EVOLIS Test Menu Inquiries:
https://www.bio-rad.com/evportal/en/US/evolutionPortal.portal?_nfpb=true&_pageLabel=help_page

In what settings are fourth generation HIV Ab/Ag combination assays indicated?

Fourth generation combination HIV Ab/Ag HIV assays should be considered in geographic areas of high HIV incidence, as seroconversion can be identified earlier using combination technology. Fourth generation HIV tests are appropriate for any healthcare setting, but HIV testing resources, processing frequency, staffing requirements, linkage to care strategies, and patient volume must be considered in the decision to use fourth generation HIV testing technology.

The Pennsylvania/ MidAtlantic AIDS Education and Training Center (PA/MA AETC) at the Health Federation of Philadelphia is not affiliated with any test manufacturer. PA/MA AETC can provide technical assistance on HIV testing technology, as well as training and education on a variety of HIV-related topics. If you have questions about fourth generation HIV testing technology or general inquiries about HIV testing and education, please contact Tina Penrose using the information below.