February 2016
HIV Diagnostic Informational Updates

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This issue contains information about FDA Approved HIV Diagnostic assays including: ADVIA Centaur® HIV Ag/Ab Combo Assay, BioPlex® 2200 HIV Ag-Ab Assay and Geenius™ HIV 1/2 Supplemental Assay.

ADVIA Centaur® HIV Ag/Ab Combo Assay (HIV-1/2 antigen/antibody combination immunoassay)

The ADVIA Centaur® HIV Ag/Ab Combo (CHIV) Assay from Siemens Healthcare Diagnostics received FDA approval on June 19, 2015. This assay is a qualitative chemiluminescent microparticle immunoassay (CMIA) for use on the Advia Centaur and Centaur XP instrument systems. The intended use is for the simultaneous detection of HIV-1 p24 antigen and antibodies to HIV-1 (including group O) and HIV-2 in serum. The Advia Centaur Ag/Ab Combo Assay is classified as a HIV-1/2 antigen/antibody combination immunoassay.

The Advia Centaur Ag/Ab Combo Assay is approved for use in pediatric and adult populations, including pregnant women. For the pediatric population, performance data are reported for children as young as 2 years of age. Siemens reports an HIV-1 specificity of 99.72% for low-risk populations and 99.26% for high-risk populations tested using the Advia Centaur Ag/Ab Combo Assay. As with other screening tests that are intended for use as an aid in the diagnosis of HIV infection, false reactive results can occur and a repeatedly reactive result must be followed with supplemental testing to verify a true infection.

In studies designed to assess early detection of HIV-1 using seroconversion panels, the Advia Centaur HIV Ag/Ab Combo Assay was generally comparable to the Abbott Architect HIV Ag/Ab Combo assay. [1,2] The analytical sensitivity for p24 antigen detection is reported by the manufacturer to be 9.04 pg/mL, which is within the range of other FDA-approved, laboratory-based HIV Ag/Ab Combo Immunoassays (range 9.02 to 25 pg/ml) (Table 1).

The Advia Centaur instrument systems are suitable for high throughput testing. The time-to-result is less than 1 hour and may be as short as 15 min for the Advia Centaur instrument and 18 min for the Centaur XP. An initial reactive result must be repeated in duplicate and at least one of the two replicates must be reactive (i.e. repeatedly reactive) for the result to reported as reactive. The Advia Centaur Ag/Ab Combo Assay does not distinguish between HIV-1 p24 antigen, HIV-1 antibody and HIV-2 antibody when a reactive result occurs.

BioPlex® 2200 HIV Ag-Ab Assay
(HIV-1/2 antigen/antibody combination immunoassay)

The BioPlex® 2200 HIV Ag-Ab Assay from Bio-Rad received FDA approval on July 23, 2015. This assay is a multiplex flow immunoassay for use on the fully automated BioPlex 2200 system. The intended use is for the simultaneous qualitative detection and differentiation of HIV-1 p24 antigen, HIV-1 antibodies (including groups M and O), and HIV-2 antibodies in serum or plasma. The assay methodology is similar to traditional immunoassays but allows for simultaneous detection using a mixture of four populations of dyed beads coated with a specific antibody or antigen. The detector is able to distinguish reactions to each of the specific dyed bead populations and the amount of captured antibody or antigen enabling detection and differentiation. The BioPlex 2200 HIV Ag-Ab Assay is classified as an antigen/antibody combination immunoassay.

The BioPlex 2200 HIV Ag-Ab Assay is approved for use in pediatric (>2 years) and adult populations including pregnant women. This assay has also been approved for screening plasma from organ donors when specimens are obtained while the donor's heart is still beating. The BioPlex 2200 HIV Ag/Ab assay is the only FDA-approved HIV-1/2 Ag/Ab combination assay that distinguishes between HIV-1 p24 antigen, HIV-1 antibody and HIV-2 antibody when a reactive result occurs. The BioPlex instrument is suitable for high throughput testing with the initial result available within one hour. All initially reactive specimens must be retested in duplicate and all individual HIV analytes (HIV-1 Ab, HIV-2 Ab, and HIV-1 p24 Ag) must be reported with this assay (Table 1 contains all possible final interpretations). A specimen is reactive if at least one analyte has an index greater than or equal to one ($\geq$1.00) A specimen is considered non-reactive if all analytes have indices less than one (<1.00) and no repeat testing is needed.

Bio-Rad reports an HIV specificity of 99.86%, HIV-1 Ab specificity of 99.92%, HIV-2 Ab specificity of 99.97% and HIV-1 p24 Ag specificity of 99.91% in low risk populations. In high-risk populations (repeat blood donors), the specificity was 99.93 %. As with other screening tests that are intended for use as an aid in the diagnosis of HIV infection, false reactive results can occur and a repeatedly reactive result must be followed with supplemental testing to verify a true infection.

In a small prospective study of 1505 specimens comparing the performance of the BioPlex 2200 HIV Ag/Ab assay to the Abbott Architect HIV Ag/Ab combo assay, the sensitivity of the BioPlex 2200 was 100%. [3] The specificity of BioPlex 2200 was 99.4% and of the previously identified acute HIV-1 specimens tested (n=11), all were detected by the BioPlex Assay. Bio-Rad reports the analytic sensitivity for p24 as 5.2pg/ml (range of 5.0-5.4).

Performance data is limited and further evaluations are needed to determine needed to determine how it performs in laboratory HIV diagnostic testing algorithm.[4] APHL encourages our members to share evaluations by publishing their data in peer-reviewed journals or submitting abstracts to national meetings.

Table 1: Characteristics of FDA-approved HIV Ag/Ab Combination Screening Immunoassays*

Test Name (Manufacturer)	FDA Approval	Method	Instrument/Platform Features	Specimen Types	HIV-1 p24 Ag Sensitivity[a] (range)	Result Output
Abbott ARCHITECT HIV Ag/Ab Combo Assay (Abbott Diagnostics)	2010	CMIA	Fully automated, random access (i2000SR); other available immunoassays[c]	Serum Plasma	18.39 (17.80-19.68) pg/mL	Nonreactive Reactive
GS HIV Combo Ag/Ab EIA (Bio-Rad Laboratories)	2011	EIA	Manual or semi-automated instrument (Evolis); for other available immunoassays contact your local sales representative	Serum Plasma	14.78 (13.22-15.89) pg/mL	Nonreactive Reactive
ADVIA Centaur HIV Ag/Ab Combo (Siemens)	2015	CMIA	Fully automated, random access (Centaur or Centaur XP); available immunoassays[c]	Serum	9.04 (6.1-11.4) pg/mL	Nonreactive Reactive
BioPlex 2200 HIV Ag-Ab (Bio-Rad Laboratories)	2015	Multiplex flow IA	Fully automated, random access (BioPlex); available immunoassays[c]	Serum Plasma	5.2 (5.0- 5.4) pg/mL	Nonreactive Reactive for HIV Ag-Ab *with* Reactive for HIV-1 Ag *and/or* Reactive for HIV-1 Ab *and/or* Reactive for HIV-2 Ab *or* Reactive, Undifferentiated
Determine HIV-1/2 Ag/Ab Combo[b] (Alere)	2013	Lateral flow, single-use device	N/A	Whole blood Serum Plasma	25 pg/mL	Nonreactive Ab Reactive Ag Reactive Ab Reactive & Ag Reactive

* For more information about FDA-approved HIV Screening tests for laboratory use only, please also refer to the updated table from CDC at: http://www.cdc.gov/hiv/pdf/testing/hiv-tests-laboratory-use.pdf

a) Reported by manufacturer in package insert. Manufacturers use different p24 standards for their calculations of analytic sensitivity and thus the significance between reported values cannot be accurately determined.

b) Current recommendations do not include use of Alere Determine in Step 1 of the Recommended Laboratory HIV Testing Algorithm. c) Or contact your local sales representative to determine other immunoassays of interest for the platform. Abbreviations: CMIA-Chemiluminescent microparticle immunoassay, EIA-Enzyme immunoassay micro-plate format, IA-Immunoassay.

Geenius™ HIV 1/2 Supplemental Assay (HIV-1/HIV-2 antibody differentiation immunoassay)

Geenius™ HIV 1/2 Supplemental Assay from Bio-Rad Laboratories received FDA approval on October 24, 2014 for commercial distribution of the device to clinical laboratories. The Geenius™ HIV 1/2 Supplemental Assay is a qualitative antibody differentiation immunoassay for whole blood, serum or plasma specimens. This is a single-use immunochromatographic device with two HIV-2 envelope peptides and 4 HIV-1 recombinant proteins/ peptides to detect and differentiate between HIV-1 and HIV-2 antibodies for specimens found to be repeatedly reactive by diagnostic screening procedures. The assay may also be used to confirm the presence of antibodies to HIV-1 and/or HIV-2 in pediatric subjects (children ≥ 2 years of age) and adults.

The Geenius™ HIV 1/2 Supplemental Assay is similar to the Multispot HIV-1/HIV-2 differentiation rapid test which was the only FDA-approved differentiation test at the time of the development of the laboratory HIV diagnostic testing algorithm.[4] Geenius not only detects and differentiates antibodies to HIV-1 and HIV-2 but is also intended to confirm the presence of these antibodies in specimens repeatedly reactive by diagnostic screening tests.

At the time of publication very few independent studies have been published examining the performance characteristics of the Geenius™ assay, particularly in comparison to Multispot. A single study published in 2013 performed a direct comparison and found that Geenius™ was a suitable alternative to the Multispot in the current laboratory algorithm (4th generation Ag/Ab immunoassay and antibody differentiation assay).[5] In that study, overall performance for both Multispot and Geenius was high: sensitivity (100%, 100%), specificity (96.3%, 99.1% but not statistically significant), and ability to differentiate HIV-1 (99.2%, 100%) and HIV-2 (98.1%, 98.1%) antibodies.[6] One study compared Geenius to INNO-LIA and found that Geenius had greater sensitivity (83%, 86%) and specificity (91%, 99%).[6] In a small study, 100% (70/70) dried blood spot specimens from HIV-1 rapid test positive patients were positive by Geenius.[7] Lastly, a poster presented at CROI 2015 demonstrated a 99.7% agreement between Geenius and HIV-1 positive samples identified with the current laboratory algorithm. The Geenius assay had fewer samples requiring additional testing due to HIV-2 cross-reactivity than Multispot.[8] In that study, repeat Geenius testing for specimens classified as HIV-2 indeterminate resolved some, but not all, specimens as HIV negative.

As previously mentioned, there is very little performance data on the Geenius assay compared to Multispot. Multispot will be discontinued in July 2016, moved up from the planned discontinuation in December 2016. Laboratories will need to replace Multispot with Geenius. In addition to the clinical trial data submitted to FDA, CDC is currently evaluating the existing data on the performance of the Geenius assay within the algorithm to provide guidance on interpretation of the additional results and these results will be shared publicly once analyses are completed. APHL encourages our members to share evaluations by publishing their data in peer-reviewed journals or submitting abstracts to national meetings.

Table 2: Comparison of HIV-1/HIV-2 antibody differentiation immunoassays

	MultiSpot (Bio-Rad Laboratories)	Geenius (Bio-Rad Laboratories)
HIV-1 peptides HIV-1 recombinant (r) proteins	gp41 (ENV) r-gp41 (ENV)	p31 (POL), gp41 (ENV) r-gp160(ENV), r-p24 (GAG)
HIV-2 peptides	gp36 (ENV)	gp36 (ENV), gp140 (ENV)
Possible Results for each assay	Nonreactive	Nonreactive
	Reactive: HIV-1 positive	Reactive: HIV-1 positive
	Reactive: HIV-2 positive	Reactive: HIV-2 positive
	No Equivalent	Reactive: HIV-2 positive with HIV-1 cross reactivity
	Reactive: HIV positive (undifferentiated)	Reactive: HIV positive untypable (undifferentiated)
	Indeterminate: HIV-1 indeterminate	Indeterminate: HIV-1 indeterminate
	No Equivalent	Indeterminate: HIV-2 indeterminate
	No Equivalent	Indeterminate: HIV indeterminate
	Invalid	Invalid
Reading & interpretation of result	Manual reading and interpretation	Geenius Reader and automatic interpretation on Geenius Software
Data Input to LIS/LIMS	Manual	Bi-directional connection to LIS/LIMS
Possible resolution of undifferentiated results	Dilution protocol (PI)	No Equivalent
Specimen type	Serum or plasma	Serum, plasma, fingerstick or Venous whole blood
Intended use	Differentiation of HIV-1 and HIV-2 antibodies in multi-test algorithm	Confirmation and differentiation of HIV-1 and HIV-2 antibodies
*For more information about FDA-approved HIV Supplemental tests, please also refer to the updated tables from CDC at: http://www.cdc.gov/hiv/pdf/testing/supplemental-hiv-tests-laboratory-use.pdf		

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