Alinity i HIV Ag/Ab (HIV Ag/Ab)-17

Prepared by: Yusra Othman /Director/Supervisor-Chem **Date:** May/24/2024
signature/title

Reviewed by: Jordan Dillard /Instructor **Date:** June 26 2024
signature/title

Approved by: Sanford N. Bailey, M.D /Chairman **Date:** June 28 2024
signature/title

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INTENDED USE

The Alinity i HIV Ag/Ab Combo assay is a chemiluminescent microparticle immunoassay (CMIA) used for the **simultaneous** qualitative detection of human immunodeficiency **virus (HIV) p24 antigen** and antibodies to **HIV type 1** (HIV-1 group M and group O) and/or type 2 (HIV-2) in human serum and plasma (EDTA and heparin) on the Alinity i analyzer.

The Alinity i HIV Ag/Ab Combo assay is intended to be used as an aid in the diagnosis of HIV-1/HIV-2 infection, including acute or primary HIV-1 infection. The assay may also be used as an aid in the diagnosis of HIV-1/HIV-2 infection in pediatric subjects (*i.e.*, children as young as two years of age) and in pregnant women.

An Alinity i HIV Ag/Ab Combo reactive result does not distinguish between the detection of

HIV-1 p24 antigen, HIV-1 antibody, or HIV-2 antibody.

The Alinity i HIV Ag/Ab Combo assay is not intended for use in screening blood or plasma donors. The effectiveness of the Alinity i HIV Ag/Ab Combo assay for use in screening blood or plasma donors has not been established. However, this assay can be used as a blood donor screening assay in urgent situations where traditional licensed blood donor screening tests are unavailable or their use is impractical.

SUMMARY AND EXPLANATION OF THE TEST

Acquired immunodeficiency syndrome (AIDS) is caused by two types of human immunodeficiency viruses, collectively designated HIV.[1], [2], [3], [4] HIV is transmitted by sexual contact, exposure to blood or blood products, and prenatal or perinatal infection of a fetus or newborn.[5] Antibodies against HIV are nearly always detected in AIDS patients and HIV-infected asymptomatic individuals.[5], [6]

Phylogenetic analysis classifies HIV type 1 (HIV-1) into groups M (major), N (non-M, non-O), O (outlier), and P.[7], [8], [9], [10] HIV-1 group M is composed of genetic subtypes (A-D, F-H, J, and K) and circulating recombinant forms (CRFs).[8], [11] Group M viruses have spread throughout the world to cause the global AIDS pandemic. However, the geographic distribution and regional predominance of HIV-1 subtypes and CRFs vary.[12] HIV-1 subtype B is the predominant subtype in North America, South America, Europe, Japan, and Australia, although other subtypes and CRFs are present in these regions as well.[12] A significant percentage of new HIV-1 infections in Europe are caused by non-B subtype strains.[13], [14] All subtypes and many recombinant strains exist in Africa.[12] In Asia, subtypes B and C, and CRF01_AE (formerly called subtype E) are found.[12] HIV-1 groups N, O, and P are endemic to west central Africa and are relatively rare.[7], [9], [10], [15], [16] However, group O infections have been identified in Europe and the USA.[14], [17], [18]

HIV type 2 (HIV-2) is similar to HIV-1 in its structural morphology, genomic organization, cell tropism, *in vitro* cytopathogenicity, transmission routes, and ability to cause AIDS.[4] HIV-2 is endemic to West Africa, but HIV-2 infections have been identified in North America and Europe at a low frequency compared to HIV-1.[14], [19], [20], [21]

Early after infection with HIV-1, but prior to seroconversion, HIV-1 core protein, p24 antigen, may be detected in HIV-1-infected individuals.[22] Alinity i HIV Ag/Ab Combo uses anti-HIV-1 p24 antibodies as reagents to detect HIV-1 p24 antigen, thereby decreasing the window period and improving early detection of HIV infection.

The key immunogenic protein for serodetection of HIV infection is the viral transmembrane protein (TMP). Antibodies against the TMP are consistently among the first to appear during seroconversion of HIV-infected individuals and remain relatively strong throughout the asymptomatic and symptomatic stages of HIV infection.[6], [23], [24] Alinity i HIV Ag/Ab Combo detects antibodies to HIV-1 groups M and O, and HIV-2 through the use of five recombinant proteins and two synthetic peptides derived from native TMP sequences of HIV-1 groups M and O, and HIV-2.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

This assay is a two-step immunoassay for the qualitative detection of HIV-1 p24 antigen and antibodies to HIV-1 (group M and group O), and HIV-2 in human serum or plasma using chemiluminescent microparticle immunoassay (CMIA) technology.

Sample, paramagnetic microparticles, assay diluent, and wash buffer are combined and incubated. The HIV-1 p24 antigen and HIV-1/HIV-2 antibodies present in the sample bind to the HIV-1/HIV-2 antigen and HIV-1 p24 monoclonal (mouse) antibody coated microparticles. The mixture is washed. Acridinium-labeled conjugate is added to create a reaction mixture and incubated. The bound HIV-1 p24 antigen and HIV-1/HIV-2 antibodies bind to the acridinium-labeled conjugates. Following another wash cycle, Pre-Trigger and Trigger solutions are added.

The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a relationship between the amount of HIV antigen and antibodies in the sample and the RLUs detected by the system optics.

The presence or absence of HIV-1 p24 antigen or HIV-1/HIV-2 antibodies in the sample is determined by comparing the chemiluminescent RLU in the reaction to the cutoff RLU determined from an active calibration.

Specimens with signal to cutoff (S/CO) values greater than or equal to 1.00 are considered reactive for HIV-1 p24 antigen or HIV-1/ HIV-2 antibodies. Specimens with S/CO values less than 1.00 are considered nonreactive for HIV-1 p24 antigen and HIV-1/ HIV-2 antibodies.

Specimens that are initially reactive in the Alinity i HIV Ag/Ab Combo assay should be retested in duplicate. Repeat reactivity is highly predictive of the presence of HIV-1 p24 antigen and/or HIV-1/HIV-2 antibodies. However, as with all immunoassays, the Alinity i HIV Ag/Ab Combo assay may yield nonspecific reactions due to other causes, particularly when testing in low prevalence populations. A repeatedly reactive specimen should be investigated further with supplemental confirmatory HIV-specific tests, such as immunoblots, antigen tests, and HIV nucleic acid tests. Supplemental testing of repeatedly reactive specimens obtained from individuals with HIV infection usually confirms the presence of HIV antibodies, HIV antigen, or HIV nucleic acid. A full differential diagnostic work-up for the diagnosis of AIDS and AIDS-related conditions includes an examination of the patient's immune status and a clinical history.

For additional information on system and assay technology, refer to the Alinity ci-series Operations Manual, Section 3.

REAGENTS

Kit Contents

Alinity i HIV Ag/Ab Combo 08P07

Volumes (mL) listed in the table below indicate the volume per cartridge.

REF	08P0721	08P0731
Tests per cartridge	100	600
Number of cartridges per kit	2	2
Tests per kit	200	1200
MICROPARTICLES	6.6 mL	32.1 mL
CONJUGATE	6.1 mL	31.6 mL
ASSAY DILUENT	6.3 mL	31.8 mL

MICROPARTICLES HIV-1/HIV-2 antigen and HIV p24 antibody (mouse IgG, monoclonal) coated microparticles in TRIS buffered saline. Minimum activity with PC1: 1.20 S/CO; minimum activity with PC2: 1.52 S/CO; minimum activity with PC3: 1.87 S/CO; minimum activity with PC4: 1.23 S/CO. Preservative: sodium azide.

CONJUGATE Acridinium-labeled HIV-1 antigens, acridinium-labeled HIV-1/HIV-2 synthetic peptides, and acridinium-labeled HIV p24 antibody (mouse IgG, monoclonal) conjugates in phosphate buffer with protein (bovine serum albumin) additive and surfactant. Minimum concentration: 61.518 ng/mL. Preservative: sodium azide.

ASSAY DILUENT TRIS buffer with protein (mouse serum and IgG) additive and surfactant. Preservative: sodium azide.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use
- Rx ONLY

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[25, 26, 27, 28]

The following warnings and precautions apply to: ASSAY DILUENT	
	
WARNING	Contains polyethylene glycol octylphenyl ether (Triton X-100) and sodium azide.
H319	Causes serious eye irritation.

EUH032	Contact with acids liberates very toxic gas.
Prevention	
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313	If eye irritation persists: Get medical advice / attention.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: MICROPARTICLES / CONJUGATE	
Contains sodium azide.	
EUH032	Contact with acids liberates very toxic gas.
P501	Dispose of contents / container in accordance with local regulations.

Safety Data Sheets are available at www.abbottdiagnostics.com or/and SDS folder.

For a detailed discussion of safety precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 8.**

Reagent Handling

Upon receipt, gently invert the unopened reagent kit by rotating it over and back for a full 180 degrees, 5 times with green label stripe facing up and then 5 times with green label stripe facing down. This ensures that liquid covers all sides of the bottles within the cartridges. During reagent shipment, microparticles can settle on the reagent septum.

- Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.
- After mixing, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- If a reagent cartridge is dropped, place in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.

- Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

For a detailed discussion of reagent handling precautions during system operation, refer to the Alinity ci-series Operations Manual, Section 7.

Reagent Storage

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright, gently invert the cartridge 10 times and place in an upright position for 1 hour before use.
Onboard	System Temperature	30 days	
Opened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright during storage, discard the cartridge. Do not reuse original reagent caps or replacement caps due to the risk of contamination and potential to compromise reagent performance.

Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright.

For information on unloading reagents, **refer to the Alinity ci-series Operations Manual, Section 5.**

Indications of Reagent Deterioration

Deterioration of the reagents may be indicated when:

- a calibration error occurs
- or a control value is out of the specified range

Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary.

For troubleshooting information, refer to the Alinity ci-series Operations Manual, Section 10.

INSTRUMENT PROCEDURE

The Alinity i HIV Ag/Ab Combo assay file must be installed on the Alinity i analyzer prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, refer to the Alinity ci-series Operations Manual, Section 2.

For information on printing assay parameters, refer to the Alinity ci-series Operations Manual, Section 5.

For a detailed description of system procedures, refer to the Alinity ci-series Operations Manual.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen types listed below were verified for use with this assay.

Other specimen types and collection tube types have not been verified with this assay.

Specimen Types	Collection Tubes
Serum	Serum, glass
	Serum, plastic
	Serum separator, plastic
Plasma	Tripotassium (K_3) EDTA, glass
	Dipotassium (K_2) EDTA, plastic
	Dipotassium (K_2) EDTA gel separator, plastic
	Disodium (Na_2) EDTA, glass
	Lithium heparin gel separator, plastic
	Sodium heparin, plastic

- Although heparin tube types will demonstrate higher S/CO values than other tube types for specimens containing HIV antibody, there is no change to the interpretation of results. Specimens that do not contain HIV antibody do not demonstrate higher S/CO values in heparin tube types.
- For blood screening in urgent situations, do not use samples collected directly from whole

blood bags as they contain anticoagulants other than EDTA and heparin.

Specimen Conditions

Do not use:

- heat-inactivated specimens
- pooled specimens
- grossly hemolyzed specimens
- specimens with obvious microbial contamination

- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Specimens should be free of bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

To ensure consistency in results, recentrifuge specimens prior to testing if

- they contain fibrin, red blood cells, or other particulate matter.

NOTE: If fibrin, red blood cells, or other particulate matter are observed, mix by low speed vortex or by inverting 10 times prior to recentrifugation.

Prepare frozen specimens as follows:

- Frozen specimens must be **completely** thawed before mixing.
- Mix thawed specimens **thoroughly** by low speed vortex or by inverting 10 times.
- Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous.
- **If specimens are not mixed thoroughly, inconsistent results may be obtained.**
- Recentrifuge specimens.

Recentrifugation of Specimens

- Transfer specimens to an appropriate tube and centrifuge at a minimum of 100 000 g-minutes.

· Examples of acceptable time and force ranges that meet this criterion are listed in the table below.

Centrifugation time using alternate RCF values can be calculated using the following formula:

$$\text{Minimum Centrifugation time (minutes)} = \frac{100\,000 \text{ g-minutes}}{\text{RCF}}$$

Recentrifugation Time (Minutes)	RCF (x g)	g-Minutes
10	10 000	100 000
20	5000	100 000
40	2500	100 000

$$RCF = 1.12 \times r_{max} (rpm/1000)^2$$

RCF -	The relative centrifugal force generated during centrifugation.
rpm -	The revolutions per minute of the rotor on which the specimens are being spun (usually the digital readout on the centrifuge will indicate the rpm).
Centrifugation Time -	The time should be measured from the time the rotor reaches the required RCF or rpm to the time it begins decelerating.
r_{max} -	Radius of the rotor in millimeters. NOTE: If custom tube adapters (i.e., adapters not defined by the centrifuge manufacturer) are used, then the radius (r_{max}) should be manually measured in millimeters and the RCF calculated.
g-minutes -	The unit of measure for the product of RCF (× g) and centrifugation time (minutes).

· Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.

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Specimen Storage

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Serum/Plasma	Room temperature	3 days	Specimens may be stored on or off the clot, red blood cells, or separator gel.
	2 to 8°C	7 days	Specimens may be stored on or off the clot, red blood cells, or separator gel.

If testing will be delayed more than 7 days, the specimens should be removed from the clot, red blood cells, or separator gel, and the serum or plasma should be stored frozen (-20°C or colder).

Avoid more than 5 freeze/thaw cycles.

Specimen Shipping

- Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

PROCEDURE

Materials Provided

08P07 Alinity i HIV Ag/Ab Combo Reagent Kit

Materials Required but not Provided

- Alinity i HIV Ag/Ab Combo assay file
- 08P0702 Alinity i HIV Ag/Ab Combo Calibrator
- 08P0712 Alinity i HIV Ag/Ab Combo Controls or other control material
- Alinity Trigger Solution
- Alinity Pre-Trigger Solution
- Alinity i-series Concentrated Wash Buffer

For information on materials required for operation of the instrument, refer to the Alinity ci-series Operations Manual, Section 1.

For information on materials required for maintenance procedures, refer to the Alinity ci-series Operations Manual, Section 9.

Assay Procedure

For a detailed description of how to run an assay, refer to the Alinity ci-series Operations

Manual, Section 5.

- If using primary or aliquot tubes, refer to the Alinity ci-series Operations Manual, Section 4 to ensure sufficient specimen is present.
- To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.

Maximum number of replicates sampled from the same sample cup: 10

Priority:

- Sample volume for first test: 150 µL
- Sample volume for each additional test from same sample cup: 100 µL

≤ 3 hours on the reagent and sample manager:

- Sample volume for first test: 150 µL
- Sample volume for each additional test from same sample cup: 100 µL

> 3 hours on the reagent and sample manager:

- Replace with a fresh aliquot of sample (patient specimens, controls, and calibrator).

- Refer to the Alinity i HIV Ag/Ab Combo calibrator package insert and/or Alinity i HIV Ag/Ab Combo control package insert for preparation and usage.
- For general operating procedures, refer to the Alinity ci-series Operations Manual, Section 5.
- For optimal performance, it is important to perform routine maintenance as described in the Alinity ci-series Operations Manual, **Section 9. Perform maintenance more frequently when required by laboratory procedures.**

Sample Dilution Procedures

Samples cannot be diluted for the Alinity i HIV Ag/Ab Combo assay.

Calibration

For instructions on performing a calibration, **refer to the Alinity ci-series Operations Manual, Section 5.**

Each assay control must be tested to evaluate the assay calibration.

Once a calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:

- A reagent kit with a new lot number is used.

Daily quality control results are outside of statistically-based quality control limits used to monitor and control system performance, as described in the Quality Control Procedures

section of this package insert.

- If statistically-based quality control limits are not available, **then the calibration should not exceed a 30-day limit for recalibration frequency.**

This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

Quality Control Procedures

The recommended control requirement for the Alinity i HIV Ag/Ab Combo assay is that a single sample of each control level be tested once every day testing performed.

To establish statistically-based control limits, each laboratory should establish its own concentration target and ranges for new control lots at each clinically relevant control level. This can be accomplished by assaying **a minimum of 20 replicates over several (3-5) days** and using the reported results to establish the expected average (target) and variability about this average (range) for the laboratory. Sources of variation that should be included in this study in order to be representative of future system performance include:

- Multiple stored calibrations
- Multiple reagent lots
- Multiple calibrator lots
- Multiple processing modules (if applicable)
- Data points collected at different times of the day

Refer to published guidelines for information or general control recommendation, for example Clinical and Laboratory Standards Institute (CLSI) Document C24-A3 or other published guidelines, for general quality control recommendations.[29]

- If quality control results do not meet the acceptance criteria defined by laboratory QC procedure, sample results may be suspect. Follow the established quality control procedures to troubleshoot. Recalibration may be necessary. For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10.**
- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

When used as a blood donor screening test in urgent situations, controls should be run with the specimens.

Quality Control Guidance

Refer to “Basic QC Practices” by James O Westgard, Ph.D. for guidance on laboratory quality control practices.[30]

Verification of Assay Claims

For protocols to verify package insert claims, refer to Verification of Assay Claims in the Alinity ci-series Operations Manual.

RESULTS

Calculation

The Alinity i analyzer calculates results for the Alinity i HIV Ag/Ab Combo assay using the ratio of the sample RLU to the cutoff RLU (S/CO) for each specimen and control.

Cutoff RLU = Calibrator 1 Mean RLU x 0.40

The cutoff RLU is stored for each reagent lot calibration.

S/CO = Sample RLU/Cutoff RLU

Interpretation of Results

The cutoff is 1.00 S/CO.

Initial Results

S/CO	Instrument Interpretation	Retest Procedure
< 1.00	Nonreactive	No retest required.
≥ 1.00	Reactive	Retest in duplicate.

Final Interpretation

Initial Interpretation	Results with Retest	Final Interpretation
Nonreactive	No retest required.	Nonreactive. HIV-1 p24 Ag and HIV-1/HIV-2 Ab not detected
Reactive	If both retest results are < 1.00	Nonreactive. HIV-1 p24 Ag and HIV-1/HIV-2 Ab not detected
	If one or both retest results are ≥ 1.00	Reactive. Presumptive evidence of HIV-1 p24 Ag and/or HIV-1/HIV-2 Ab; perform supplemental confirmatory assay(s)

A specimen with a final result of reactive should be investigated further with supplemental confirmatory HIV-specific tests, **such as immunoblots, antigen tests, and HIV nucleic acid tests.**

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, **refer to the Alinity ci-series Operations Manual, Section 5.**

LIMITATIONS OF THE PROCEDURE

- The interpretation of specimens with a final result of reactive by the Alinity i HIV Ag/Ab Combo assay and indeterminate by supplemental testing is not definitive; further clarification may be obtained by testing another specimen taken **at least 1 month later**.[31]
- The Alinity i HIV Ag/Ab Combo assay result and supplemental assay results should be interpreted in conjunction with the patient's clinical presentation, history, and other laboratory results. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- An individual who has antibodies to HIV is presumed to be infected with the virus; however, an individual who has participated in an HIV vaccine study may develop antibodies to the vaccine and may or may not be infected with HIV. Clinical correlation is indicated with appropriate counseling, medical evaluation, and possibly additional testing to determine whether a diagnosis of HIV infection is accurate.
- A test result that is nonreactive does not exclude the possibility of exposure to or infection with HIV-1 and/or HIV-2. Nonreactive results in this assay for individuals with prior exposure to HIV-1 and/or HIV-2 may be due to antigen and antibody levels that are below the limit of detection of this assay.
- The performance of this assay has not been established for individuals younger than 2 years of age. Nearly all infants born to HIV-infected mothers passively acquire maternal antibody and, in some cases, will test antibody positive until age 18 months regardless of whether they are infected. Definitive diagnosis of HIV infection in early infancy requires other assays, including HIV nucleic acid tests or viral culture.[32]
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).[33, 34] Such specimens may show either falsely elevated or depressed results when tested with assay kits (such as Alinity i HIV Ag/Ab Combo) that employ mouse monoclonal antibodies.[33] Alinity i HIV Ag/Ab Combo reagents contain a component that reduces the effect of HAMA reactive specimens.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference, and anomalous values may be observed. Additional information may be required for diagnosis.[35]
- Refer to the SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS section of this package insert for specimen limitations.

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

The Alinity i analyzer and the ARCHITECT i System utilize the same reagents and sample/reagent ratios.

Alinity i Analyzer Specific Studies

The following results were generated using the Alinity i analyzer.

Precision

Within-Laboratory Precision

A study was performed based on guidance from CLSI EP05-A3.[36] Testing was conducted using 1 lot of Alinity i HIV Ag/Ab Combo Reagent Kit, 1 lot of HIV Ag/Ab Combo Calibrator, and 1 lot of HIV Ag/Ab Combo Controls and 1 instrument. Five controls and 12 panels were assayed in a minimum of 2 replicates at 2 separate times per day on 20 different days.

Sample	n	Mean (S/CO)	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
			SD	%CV	SD	%CV
Negative Control	120	0.06	0.009	NA	0.011	NA
Positive Control 1	119	5.86	0.175	3.0	0.176	3.0
Positive Control 2	120	4.07	0.153	3.8	0.163	4.0
Positive Control 3	119	3.01	0.074	2.5	0.086	2.9
Positive Control 4	119	2.07	0.058	2.8	0.069	3.3
HIV-1 High Negative Panel	120	0.90	0.035	3.9	0.035	3.9
HIV-1 Low Positive Panel	120	1.22	0.049	4.0	0.053	4.3
HIV-1 High Positive Panel	119	10.47	0.326	3.1	0.359	3.4
HIV-2 High Negative Panel	120	0.83	0.029	3.5	0.029	3.5
HIV-2 Low Positive Panel	120	1.13	0.041	3.7	0.046	4.1
HIV-2 High Positive Panel	120	12.73	0.481	3.8	0.596	4.7
HIV p24 High Negative Panel	120	0.78	0.029	3.7	0.033	4.2
HIV p24 Low	120	1.16	0.047	4.0	0.048	4.1

Sample	n	Mean (S/CO)	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
			SD	%CV	SD	%CV
Positive Panel						
HIV p24 Moderate Positive Panel	120	2.40	0.069	2.9	0.087	3.6
HIV p24 High Positive Panel	120	9.54	0.417	4.4	0.417	4.4
HIV-1 gO High Negative Panel	120	0.82	0.029	3.5	0.031	3.8
HIV-1 gO Low Positive Panel	119	1.22	0.049	4.0	0.050	4.1

[a]Includes within-run, between-run, and between-day variability.

System Reproducibility

A study was performed based on guidance from CLSI EP05-A2 and CLSI EP15-A2.*37*, *38* Testing was conducted at 3 clinical sites using 1 lot of the Alinity i HIV Ag/Ab Combo Reagent Kit, 1 lot of the Alinity i HIV Ag/Ab Combo Calibrator, and 1 lot of the Alinity i HIV Ag/Ab Combo Controls and 1 instrument per site. Five controls and 8 panels were assayed in replicates of 4 at 2 separate times per day for 5 days.

Sample	n	Mean (S/CO)	Within-Run (Repeatability)		Within-Day[a]		Within-Laboratory Precision (Total)[b]		Overall Reproducibility[c]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Control	120	0.06	0.008	NA	0.008	NA	0.012	NA	0.013	NA
Positive Control 1	120	5.02	0.175	3.5	0.190	3.8	0.194	3.9	0.194	3.9
Positive Control 2[d]	120	3.05	0.065	2.1	0.082	2.7	0.082	2.7	0.085	2.8
Positive Control	120	2.89	0.068	2.4	0.075	2.6	0.077	2.7	0.136	4.7

Sample	n	Mean (S/CO)	Within-Run (Repeatability)		Within-Day[a]		Within-Laboratory Precision (Total)[b]		Overall Reproducibility[c]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
3										
Positive Control 4	120	1.86	0.056	3.0	0.060	3.2	0.061	3.3	0.061	3.3
High Negative anti-HIV-1	120	0.86	0.025	2.8	0.026	3.1	0.026	3.1	0.032	3.7
Low Positive anti-HIV-1	120	1.40	0.047	3.3	0.055	3.9	0.055	3.9	0.055	3.9
High Negative anti-HIV-2	120	0.61	0.017	2.8	0.019	3.2	0.022	3.6	0.023	3.8
Low Positive anti-HIV-2	120	1.10	0.030	2.7	0.033	3.0	0.033	3.0	0.036	3.3
High Negative anti-HIV p24	120	0.71	0.022	3.1	0.023	3.2	0.023	3.3	0.024	3.4
Low Positive anti-HIV p24	120	1.10	0.029	2.6	0.030	2.8	0.036	3.3	0.036	3.3
High Negative anti-HIV-1 gO	120	0.63	0.019	3.0	0.019	3.0	0.022	3.5	0.024	3.8
Low Positive	120	1.14	0.030	2.6	0.033	2.9	0.033	2.9	0.037	3.3

Sample	n	Mean (S/CO)	Within-Run (Repeatability)		Within-Day[a]		Within-Laboratory Precision (Total)[b]		Overall Reproducibility[c]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
anti-HIV-1 gO										

[a] Includes within-run and between-run variability.

[b] Includes within-run, between-run, and between-day variability.

[c] Includes within-run, between-run, between-day and between-site variability.

[d] An outlying run was observed. Based on guidance from CLSI EP05-A2, a replacement run was performed and the results are shown in the table above. Without the replacement run, the within-run (repeatability) %CV was 43.1%, the within-day %CV was 43.2%, the within-laboratory precision (total) %CV was 43.3%, and the overall reproducibility %CV was 43.3%.

Antigen Analytical Sensitivity

The analytical sensitivity of the Alinity i HIV Ag/Ab Combo assay was evaluated on the Alinity i analyzer. Antigen sensitivity was conducted using 3 lots of Alinity i HIV Ag/Ab Combo Reagent Kit with the the Bio-Rad HIV-1 Antigen Standard. The HIV-1 p24 antigen analytical sensitivity results ranged **from 20.41 pg/mL to 20.81 pg/mL**.

Seroconversion Sensitivity

To determine the seroconversion sensitivity, 37 seroconversion panels obtained from commercial vendors were tested on the Alinity i analyzer using the Alinity i HIV Ag/Ab Combo assay. The panel results were evaluated against ARCHITECT HIV Ag/Ab Combo assay and data are summarized in the following table.

Vendor ID	Number of Panel Members	Alinity i HIV Ag/Ab Combo		ARCHITECT HIV Ag/Ab Combo		Difference in Days
		Number of Reactive Panel Members	Number of Days to First Reactive Result	Number of Reactive Panel Members	Number of Days to First Reactive Result	
PRB916	6	3	15	3	15	0
PRB926	6	4	7	4	7	0
PRB941	6	4	9	4	9	0

Vendor ID	Number of Panel Members	Alinity i HIV Ag/Ab Combo		ARCHITECT HIV Ag/Ab Combo		Difference in Days
		Number of Reactive Panel Members	Number of Days to First Reactive Result	Number of Reactive Panel Members	Number of Days to First Reactive Result	
PRB944	6	5	2	5	2	0
PRB949	4	1	18	1	18	0
PRB952	6	4	10	4	10	0
PRB954	7	2	17	2	17	0
PRB957	7	2	23	2	23	0
PRB961	9	2	27	2	27	0
PRB962	6	2	14	2	14	0
PRB963	7	2	17	2	17	0
PRB964	6	1	22	1	22	0
PRB966	10	3	44	3	44	0
PRB969	10	3	70	3	70	0
PRB975	5	1	14	1	14	0
PRB976	4	2	7	2	7	0
PRB978	7	1	33	1	33	0
6240	13	6	23	6	23	0
6243	10	4	25	4	25	0
6244	14	2	28	2	28	0
6248	7	2	18	2	18	0
9012	8	3	16	3	16	0
9013	7	1	25	1	25	0
9015	8	2	30	2	30	0
9016	10	2	59	2	59	0
9017	11	4	24	4	24	0

		Alinity i HIV Ag/Ab Combo		ARCHITECT HIV Ag/Ab Combo		
Vendor ID	Number of Panel Members	Number of Reactive Panel Members	Number of Days to First Reactive Result	Number of Reactive Panel Members	Number of Days to First Reactive Result	Difference in Days
9018	11	3	28	3	28	0
9020	22	3	90	3	90	0
9021	17	4	47	4	47	0
9024	12	1	53	1	53	0
9027	9	5	14	5	14	0
9030	16	3	47	3	47	0
9032	14	7	24	7	24	0
9034	13	3	46	3	46	0
9076	9	3	66	3	66	0
9089	6	3	16	3	16	0
12008	13	5	28	5	28	0

Percent Agreement

Studies were performed to compare the HIV Ag/Ab Combo assay on the Alinity i analyzer and the ARCHITECT i2000SR system using 1 lot each of the HIV Ag/Ab Combo Reagent Kit, HIV Ag/Ab Combo Calibrator, and HIV Ag/Ab Combo Controls.

Percent Agreement was evaluated by testing a total of 432 HIV negative and positive specimens (Panel 1) consisting of the following HIV subpopulations: HIV negative, HIV-1 antibody positive, HIV-2 antibody positive, and HIV Ag viral isolates. An aliquot of each specimen was tested on 1 Alinity i analyzer at each of the 3 clinical testing sites and on 1 ARCHITECT i2000SR instrument at 1 clinical testing site.

Percent agreement was also evaluated for the following populations: known HIV negative donors, pregnant females, and pediatrics. An aliquot of each specimen was tested on 1 Alinity i analyzer at one of the 3 clinical testing sites and compared to results obtained on 1 ARCHITECT i2000SR instrument.

Panel 1

There were 432 total specimens tested.

Specimen Category	Site	Alinity i HIV Ag/Ab Combo	ARCHITECT HIV Ag/Ab Combo		NPA[a] (95% CI[a])	PPA[a] (95% CI[a])	All Sites	
			Reactive	Nonreactive			NPA[a] (95% CI[a])[b]	PPA[a] (95% CI[a])[b]
HIV negative	1	Reactive	0	0	100.00% (102/102) (96.45%,100.00%)	NA	100.00%	NA
		Nonreactive	0	102				
	2	Reactive	0	0				
		Nonreactive	0	102				
	3	Reactive	0	0				
		Nonreactive	0	102				
HIV-1 Antibody Positive	1	Reactive	103	0	NA	100.00% (103/103) (96.48%,100.00%)	NA	100.00%
		Nonreactive	0	0				
	2	Reactive	102[c]	0	NA	100.00% (102/102) (96.45%,100.00%)		
		Nonreactive	0	0				
	3	Reactive	103	0	NA	100.00% (103/103) (96.48%,100.00%)		
		Nonreactive	0	0				
HIV-2 Antibody Positive	1	Reactive	102	0	NA	100.00% (102/102) (96.45%,100.00%)	NA	100.00%
		Nonreactive	0	0				
	2	Reactive	102	0	NA	100.00% (102/102) (96.45%,100.00%)		
		Nonreactive	0	0				
	3	Reactive	102	0	NA	100.00% (102/102) (96.45%,100.00%)		
		Nonreactive	0	0				
HIV Antigen Viral Isolates	1	Reactive	99	0	100.00% (26/26) (86.77%, 100.00%)	100.00% (99/99) (96.34%,100.00%)	100.00% (90.00%, 100.00%)	100.00% (97.03%,100.00%)
		Nonreactive	0	26				
	2	Reactive	97	1	96.15% (25/26) (80.36%, 99.90%)	97.98% (97/99) (92.89%,99.75%)		
		Nonreactive	2	25				
	3	Reactive	99	1	96.15% (25/26)	100.00% (99/99)		

Specimen Category	Site	Alinity i HIV Ag/Ab Combo	ARCHITECT HIV Ag/Ab Combo		NPA[a] (95% CI[a])	PPA[a] (95% CI[a])	All Sites	
			Reactive	Nonreactive			NPA[a] (95% CI[a])[b]	PPA[a] (95% CI[a])[b]
		Nonreactive	0	25	(80.36%, 99.90%)	(96.34%, 100.00%)		

[a] NPA=Negative Percent Agreement, PPA=Positive Percent Agreement, CI=Confidence Interval.

[b] 95% confidence intervals across all three sites were estimated using a bootstrap technique with 1000 iterations.

[c] One result was excluded due to insufficient sample volume for retest.

Donors

Six hundred six (606) known HIV negative specimens from a donor population were tested.

Site	Alinity i HIV Ag/Ab Combo	ARCHITECT HIV Ag/Ab Combo		NPA[a] (95% CI[a])	Overall NPA[a] (95% CI[a])
		Reactive	Nonreactive		
1	Reactive	0	0	100.00% (202/202)	
	Nonreactive	0	202	(98.19%,100.00%)	
2	Reactive	0	0	100.00% (202/202)	100.00% (606/606)
	Nonreactive	0	202	(98.19%,100.00%)	(99.39%,100.00%)
3	Reactive	0	0	100.00% (202/202)	
	Nonreactive	0	202	(98.19%,100.00%)	

[a] NPA=Negative Percent Agreement, PPA=Positive Percent Agreement, CI=Confidence Interval.

Pregnant Females

Fifty-two (52) specimens from a pregnant female population were tested.

Site	Alinity i HIV Ag/Ab Combo	ARCHITECT HIV Ag/Ab Combo		NPA[a] (95% CI[a])	PPA[a] (95% CI[a])	Overall NPA[a] (95% CI[a])	Overall PPA[a] (95% CI[a])
		Reactive	Nonreactive				
1	Reactive	2	0	100.00% (16/16)	100.00% (2/2)		
	Nonreactive	0	16	(79.41%,100.00%)	(15.81%,100.00%)		
2	Reactive	2	0	100.00% (15/15)	100.00% (2/2)	100.00% (46/46)	100.00% (6/6)
	Nonreactive	0	15	(78.20%,100.00%)	(15.81%,100.00%)	(92.29%,100.00%)	(54.07%,100.00%)
3	Reactive	2	0	100.00% (15/15)	100.00% (2/2)		
	Nonreactive	0	15	(78.20%,100.00%)	(15.81%,100.00%)		

[a] NPA=Negative Percent Agreement, PPA=Positive Percent Agreement, CI=Confidence Interval.

Pediatrics

Sixty-seven (67) specimens from a pediatric population were tested.

Site	Alinity i HIV Ag/Ab Combo	ARCHITECT HIV Ag/Ab Combo		NPA[a] (95% CI[a])	PPA[a] (95% CI[a])	Overall NPA[a] (95% CI[a])	Overall PPA[a] (95% CI[a])
		Reactive	Nonreactive				
1	Reactive	2	0	100.00% (20/20)	100.00% (2/2)		
	Nonreactive	0	20	(83.16%,100.00%)	(15.81%,100.00%)		
2	Reactive	2	0	100.00% (20/20)	100.00% (2/2)	100.00% (61/61)	100.00% (6/6)
	Nonreactive	0	20	83.16%,100.00%)	(15.81%,100.00%)	(94.13%,100.00%)	(54.07%,100.00%)
3	Reactive	2	0	100.00% (21/21)	100.00% (2/2)		
	Nonreactive	0	21	83.89%,100.00%)	(15.81%,100.00%)		

[a] NPA=Negative Percent Agreement, PPA=Positive Percent Agreement, CI=Confidence Interval.

$$\text{Positive Percent Agreement} = \frac{\text{[Number of reactive Alinity i results in agreement with ARCHITECT i2000SR results]}}{\text{[Total number of reactive ARCHITECT i2000SR results]}} \times 100\%$$

$$\text{Negative Percent Agreement} = \frac{\text{[Number of nonreactive Alinity i results in agreement with ARCHITECT i2000SR results]}}{\text{[Total number of nonreactive ARCHITECT i2000SR results]}} \times 100\%$$

ARCHITECT System Specific Studies

The following results were generated using the ARCHITECT i System.

Clinical Performance

A multicenter study was conducted to establish the performance of the ARCHITECT HIV Ag/Ab Combo assay in the following populations: individuals at low risk for HIV, known HIV-1 antigen positive samples, known HIV-1 and HIV-2 antibody positive specimens, individuals at increased risk for HIV, pregnant females, and pediatric subjects. For specimens from the low and increased risk populations that were repeatedly reactive by ARCHITECT HIV Ag/Ab Combo and/or an FDA-licensed HIV-1/2/O Antibody assay, supplemental testing was performed using FDA-licensed HIV-1 Western blot, HIV-2 EIA, and HIV-1 RNA PCR tests and using research-use-only HIV-2 Western blot and HIV-1 p24 Antigen assays.

Specificity

A total of 6164 prospectively collected specimens from a low risk for HIV population in the US (age range: 16 to 89 years) were tested by the ARCHITECT HIV Ag/Ab Combo assay and an FDA-licensed HIV-1/2/O Antibody assay. The low risk for HIV population includes individuals in a low prevalence setting,[31] apparently healthy individuals, and pregnant females in the first trimester of pregnancy.

The results for the 6164 specimens from ARCHITECT HIV Ag/Ab Combo assay are presented in Table 1.

Table 1: Reactivity of the ARCHITECT HIV Ag/Ab Combo Assay in Individuals at Low Risk for Infection with HIV

	ARCHITECT HIV Ag/Ab Combo				Repeatedly Reactive Specimens (Number Positive by Method)		
Specimen Category	Number of Specimens Tested	Number of Nonreactive Specimens (%)	Number of Initially Reactive Specimens (%)	Number of Repeatedly Reactive Specimens (%)	HIV-1 Western Blot (%)	HIV-1 p24 Antigen (%)	HIV-1 RNA PCR (%)

	ARCHITECT HIV Ag/Ab Combo				Repeatedly Reactive Specimens (Number Positive by Method)		
Specimen Category	**Number of Specimens Tested**	**Number of Nonreactive Specimens (%)**	**Number of Initially Reactive Specimens (%)**	**Number of Repeatedly Reactive Specimens (%)**	**HIV-1 Western Blot (%)**	**HIV-1 p24 Antigen (%)**	**HIV-1 RNA PCR (%)**
Individuals in Low Prevalence Setting - Serum	2663	2652 (99.59)	13 (0.49)	11 (0.41)	9 (0.34)	0 (0.00)	0 (0.00)
Individuals in Low Prevalence Setting - Plasma	2671	2631 (98.50)	41 (1.54)	40 (1.50)	27 (1.01)	1 (0.04)	1 (0.04)
Individuals in Low Prevalence Setting - Fresh Plasma[a]	580	580 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Low Risk Pregnant Females - Plasma	250	250 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Total	**6164**	**6113 (99.17)**	**54 (0.88)**	**51 (0.83)**	**36**[b]	**1**[b]	**1**[b]

[a] Not frozen prior to testing

[b] 36 specimens confirmed positive by HIV-1 Western blot, 1 specimen negative by HIV-1 Western blot was positive by both HIV-1 p24 Antigen test and HIV-1 RNA PCR.

As shown in Table 1 above, 99.17% (6113/6164) of the low risk population was nonreactive, 0.88% (54/6164) was initially reactive, and 0.83% (51/6164) was repeatedly reactive with the ARCHITECT HIV Ag/Ab Combo assay. Of the 6164 specimens, 37 were confirmed positive (36 by HIV-1 Western blot and 1 by both HIV-1 p24 antigen and HIV-1 RNA PCR). The

specificity of the ARCHITECT HIV Ag/Ab Combo assay in the low risk population in this study was 99.77% (6113/6127) with an exact 95% confidence interval of 99.62% to 99.88%.

Sensitivity

Detection of HIV-1 Antigen

HIV-1 antigen positive samples (n=63) were tested with the ARCHITECT HIV Ag/Ab Combo assay.

The 63 HIV-1 antigen positive samples included 5 antibody-negative HIV-1 antigen group M specimens (subtypes C, B and CRF02), 20 commercially available HIV-1 antibody-negative antigen panel members, and 38 unique viral isolates that were propagated in cell culture and classified as HIV-1 group M (subtypes A-D, F, G, CRF01, CRF02, and URFs) and HIV-1 group O. The sensitivity of the ARCHITECT HIV Ag/Ab Combo assay in this study was 100.00% (63/63) with an exact 95% confidence interval of 94.31% to 100.00%. The reactivity for HIV-1 antigen positive antibody-negative samples is presented in Table 2.

Table 2: Reactivity of the ARCHITECT HIV Ag/Ab Combo Assay for HIV-1 Antigen-Positive Antibody-Negative Samples

		ARCHITECT HIV Ag/Ab Combo	**Repeatedly Reactive Specimens (Number Positive by Method)**		
Specimen Category	**Number of Specimens Tested**	**Number of Repeatedly Reactive Specimens (%)**	**Direct HIV-1 p24 Antigen (%)**	**Neutralization HIV-1 p24 Antigen/Number Tested**	**HIV-1 RNA PCR (%)**
HIV-1 Antigen Specimens[a]	5	5 (100.00)	5 (100.00)	5/5	ND
HIV-1 Antigen Panel 1[a]	4	4 (100.00)	3 (75.00)	3/3	4 (100.00)
HIV-1 Antigen Panel 2[a]	16	16 (100.00)	14 (87.50)	14/14	16 (100.00)
HIV-1 Viral Isolate Panel	38	38 (100.00)	36 (94.74)[b]	21/22[c]	ND
Total	**63**	**63 (100.00)**	**58 (92.06)**	**43/44**	**20 (100.00)**

ND = not done (specimens of known subtype)

[a] Of the 25 HIV-1 antigen specimens and panel members, 17 specimens were nonreactive by the FDA-licensed HIV-1/2/O Antibody Assay, and all 25 were negative by HIV-1 Western blot.

[b] Two group O samples were nonreactive by the Direct HIV-1 p24 Antigen Assay.

[c] 14 of the 36 repeatedly reactive specimens by the Direct HIV-1 p24 Antigen Assay were not tested by neutralization due to limited volume, and one sample (group M subtype CRF01_AE) that was repeatedly reactive by the Direct HIV-1 p24 Antigen Assay did not confirm by neutralization.

Detection of HIV-1 Antigen Subtypes

Nine HIV-1 antigen group M subtype specimens and the 38 HIV-1 viral isolates were tested with the ARCHITECT HIV Ag/Ab Combo assay. The reactivity by HIV-1 antigen subtype and country of origin is presented in Table 3.

Table 3: Reactivity of the ARCHITECT HIV Ag/Ab Combo Assay for HIV-1 Antigen Group M Specimens and HIV-1 Viral Isolates (by Subtype and Country of Origin)

			ARCHITECT HIV Ag/Ab Combo	**HIV-1 p24 Tests**	
Source (# of Specimens)	**Subtype**	**Number of Specimens Tested**	**Number of Repeatedly Reactive Specimens (%)**	**Direct Antigen Repeatedly Reactive[a] (%)**	**Neutralization Antigen Positive/ Number Tested[a]**
Group M Specimens[b](9)					
S. Africa	B	2	2 (100.00)	2 (100.00)	2/2
S. Africa	C	6	6 (100.00)	6 (100.00)	6/6
Cameroon	CRF02_AG	1	1 (100.00)	1 (100.00)	1/1
Viral Isolates[a](38)					
Uganda	A	2	2 (100.00)	2 (100.00)	1/1
United States (4), Thailand (2), Brazil (1)	B	7	7 (100.00)	7 (100.00)	4/4
Uganda (2), Zambia (1), Ethiopia (1), Senegal (1),	C	6	6 (100.00)	6 (100.00)	3/3

Source (# of Specimens)	Subtype	Number of Specimens Tested	ARCHITECT HIV Ag/Ab Combo	HIV-1 p24 Tests	
			Number of Repeatedly Reactive Specimens (%)	Direct Antigen Repeatedly Reactive[a] (%)	Neutralization Antigen Positive/ Number Tested[a]
Somalia (1)					
Senegal (1), Uganda (3)	D	4	4 (100.00)	4 (100.00)	3/3
Brazil	F	4	4 (100.00)	4 (100.00)	2/2
Kenya	G	1	1 (100.00)	1 (100.00)	1/1
Côte d'Ivoire (1), Thailand (1)	URF[c]	2	2 (100.00)	2 (100.00)	1/1
Thailand (6), Indonesia (2)	CRF01_AE	8	8 (100.00)	8 (100.00)	6/7
Djibouti	CRF02_AG	2	2 (100.00)	2 (100.00)	ND
United States (1), Spain (1)	Group O	2	2 (100.00)	0 (0.00)[d]	NA
Total		**47**	**47 (100)**	**45 (95.74)**	**30/31 (96.77%)**

CRF = circulating recombinant form, URF = unique recombinant form, NA = not applicable, ND = not done

[a] Of the 38 viral isolates, 36 were repeatedly reactive by the Direct HIV-1 p24 Antigen Assay. Of the 36, 21 were confirmed by neutralization, 1 did not confirm (group M subtype CRF01_AE), and 14 were not tested by neutralization due to limited specimen volume.

[b] Of the 9 HIV-1 antigen group M specimens, 2 (subtype C) were nonreactive by the FDA-licensed HIV-1/2/O Antibody Assay and 5 were negative by HIV-1 Western blot.

[c] One viral isolate subtype was B/A/B, and one viral isolate subtype was A/G/G.

[d] Two group O samples were nonreactive by the Direct HIV-1 p24 Antigen Assay.

Detection of HIV Antibodies

A total of 1003 serum and plasma specimens were collected from HIV-infected individuals in a US population and confirmed positive for HIV-1 antibodies by Western blot and tested using the ARCHITECT HIV Ag/Ab Combo and FDA-Licensed HIV-1/2/O Antibody assays. All 1003 specimens were repeatedly reactive using the ARCHITECT HIV Ag/Ab Combo assay. The sensitivity of the ARCHITECT HIV Ag/Ab Combo assay in this study was 100.00% (1003/1003) with an exact 95% confidence interval of 99.63 to 100.00%. The results from specimens positive for antibodies to HIV-1 are presented in Table 4.

Table 4: Reactivity of the ARCHITECT HIV Ag/Ab Combo Assay in Specimens Positive for Antibodies to HIV-1

		ARCHITECT HIV Ag/Ab Combo	**FDA-Licensed HIV-1/2/O Antibody Assay**	
Specimen Category	**Number of Specimens Tested**	**Number of Repeatedly Reactive Specimens (%)**	**Number of Repeatedly Reactive Specimens (%)**	**HIV-1 Western Blot (%)**
Asymptomatic	416	416 (100.00)	416 (100.00)	416 (100.00)
Symptomatic	183	183 (100.00)	182 (99.45)	183 (100.00)
Diagnosed with AIDS	404	404 (100.00)	404 (100.00)	404 (100.00)
Total	**1003**	**1003 (100.00)**	**1002 (99.90)**[a]	**1003 (100.00)**

[a] A second aliquot for the one specimen that was nonreactive by the FDA-licensed HIV-1/2/O Antibody assay was tested and was repeatedly reactive.

A total of 201 plasma specimens confirmed positive by HIV-2 Western blot from Côte d'Ivoire were tested using the ARCHITECT HIV Ag/Ab Combo assay and FDA-licensed HIV-1/2/O Antibody assay as presented in Table 5. All 201 specimens were repeatedly reactive with the ARCHITECT HIV Ag/Ab Combo assay. The sensitivity of the ARCHITECT HIV Ag/Ab Combo assay in this study was 100.00% (201/201) with an exact 95% confidence interval of 98.18 to 100.00%.

Table 5: Reactivity of the ARCHITECT HIV Ag/Ab Combo Assay in Specimens Positive for Antibodies to HIV-2

Specimen Category	Number of Specimens Tested	ARCHITECT HIV Ag/Ab Combo	FDA-Licensed HIV-1/2/O Antibody Assay	HIV-2 Western Blot (%)
		Number of Repeatedly Reactive Specimens (%)	Number of Repeatedly Reactive Specimens (%)	
Anti-HIV-2 Positive	201	201 (100.00)	201 (100.00)	201 (100.00)

Reactivity of the ARCHITECT HIV Ag/Ab Combo Assay for Specimens with Antibodies to HIV-1 Groups M, O, and N and Antibodies to HIV-2

In an internal study, a total of 693 specimens identified as anti-HIV-1 group M, group O, and group N and anti-HIV-2 were tested. Of the 693 specimens, 500 anti-HIV-1 group M subtypes and 8 anti-HIV-1 group N specimens from Argentina, Brazil, Cameroon, Ghana, Saudi Arabia, South Africa, Thailand, Uganda, and United Kingdom were reactive by the ARCHITECT HIV Ag/Ab Combo assay. In addition, a total of 65 anti-HIV-1 group O specimens from Cameroon, Equatorial Guinea, Spain, and United States and 120 anti-HIV-2 specimens from Côte d'Ivoire were reactive by the ARCHITECT HIV Ag/Ab Combo assay. The HIV-1/HIV-2 antibody sensitivity is summarized in Table 6.

Table 6: HIV-1/HIV-2 Antibody Sensitivity

HIV Antibody Type	Subtype	ARCHITECT HIV Ag/Ab Combo Number of Repeatedly Reactive/Number Tested
HIV-1 Group M Antibody	A	54/54
	B	44/44
	C	44/44
	D	40/40
	F	24/24
	G	20/20
	CRF 01	92/92
	CRF 02	51/51
	CRF 09	2/2
	CRF 11	12/12

HIV Antibody Type	Subtype	ARCHITECT HIV Ag/Ab Combo Number of Repeatedly Reactive/Number Tested
	CRF 13	4/4
	URF	113/113
HIV-2 Antibody	NA	120/120
HIV-1 Group O Antibody	NA	65/65
HIV-1 Group N Antibody	NA	8/8
Total		**693/693**

CRF = circulating recombinant form; URF = unique recombinant form; NA = not applicable

Reactivity in Individuals at Increased Risk for HIV Infection

A total of 693 specimens from individuals in a US population at increased risk for infection with HIV were tested using the ARCHITECT HIV Ag/Ab Combo assay and an FDA-licensed HIV-1/2/O Antibody assay. The specimens were collected from individuals (age range: 18 to 66 years) with one or more of the following risk factors: injecting drug users, unprotected sex with someone who is infected with HIV, diagnosed or treated for a sexually transmitted disease (STD), hepatitis or tuberculosis, multiple sex partners, men who have sex with men, unprotected sex with someone who has been diagnosed or treated for an STD, and risk factor not identified but requested an HIV test.

Table 7: Reactivity of the ARCHITECT HIV Ag/Ab Combo Assay in Individuals at Increased Risk for Infection with HIV

		Number of Repeatedly Reactive Specimens (%)		Repeatedly Reactive Specimens (Number Reactive/Positive by Method)				
Specimen Category	Number of Specimens Tested	ARCHITECT HIV Ag/Ab Combo	FDA-Licensed HIV-1/2/O Antibody Assay	HIV-1 Western Blot (%)	HIV-2 EIA (%)	HIV-2 Western Blot (%)	HIV-1 p24 Antigen (%)	HIV-1 RNA PCR (%)
Individuals at Increased Risk for HIV - US Population	693	71 (10.25)	77 (11.11)	65 (9.38)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)

Of the 693 specimens from individuals at increased risk in the US, 71 (10.25%) were repeatedly reactive by the ARCHITECT HIV Ag/Ab Combo assay. Of these 71 specimens, 65 were confirmed positive by HIV-1 Western blot, and all 65 were also repeatedly reactive by the FDA-licensed assay.

A comparison of the ARCHITECT HIV Ag/Ab Combo results versus the FDA-licensed HIV-1/2/O Antibody assay results is summarized in Table 8. None of the specimens that were repeatedly reactive only on ARCHITECT HIV Ag/Ab Combo or only on the FDA-licensed Antibody assay were confirmed positive.

Table 8: ARCHITECT HIV Ag/Ab Combo and FDA-Licensed HIV-1/2/O Antibody Assay Results in Individuals at Increased Risk for Infection with HIV

ARCHITECT HIV Ag/Ab Combo	FDA-Licensed HIV-1/2/O Antibody Assay		
	Repeatedly Reactive	**Nonreactive**	**Total**
Repeatedly Reactive	69	2[a]	71
Nonreactive	8[b]	614	622
Total	**77**	**616**	**693**

[a] Both specimens were negative by HIV-1 Western blot, HIV-2 EIA, HIV-1 p24 Antigen Assay, and HIV-1 RNA PCR.

[b] Seven specimens were negative by HIV-1 Western blot, and 1 specimen was HIV-1 Western blot indeterminate.

All 8 specimens were negative by HIV-2 EIA, HIV-1 p24 Antigen Assay and HIV-1 RNA PCR.

Reactivity in Individuals at Increased Risk for Infection with HIV-2

A total of 513 specimens from individuals at increased risk for infection with HIV from an HIV-2 endemic area (Côte d'Ivoire) were tested by the ARCHITECT HIV Ag/Ab Combo assay and an FDA-licensed HIV-1/2/O Antibody assay. The specimens were collected from individuals (age range: 17 to 60 years) with one or more of the following risk factors: unprotected sex with someone who is infected with HIV, multiple sex partners, men who have sex with men, and injecting drug users.

Table 9: Reactivity of the ARCHITECT HIV Ag/Ab Combo Assay in Individuals at Increased Risk for Infection with HIV-2

Specimen Category	Number of Specimens Tested	Number of Repeatedly Reactive Specimens (%)		Repeatedly Reactive Specimens (Number Reactive/Positive by Method)				
		ARCHITECT HIV Ag/Ab Combo	FDA-Licensed HIV-1/2/O Antibody Assay	HIV-1 Western Blot (%)	HIV-2 EIA (%)	HIV-2 Western Blot (%)	HIV-1 p24 Antigen (%)	HIV-1 RNA PCR (%)
Individuals at Increased Risk from an HIV-2 Endemic Area	513	89 (17.35)	87 (16.96)	79 (15.40)[a]	82 (15.98)	24 (4.68)[b]	1 (0.19)[c]	1 (0.19)[c]

[a] Specimens were repeatedly reactive by ARCHITECT HIV Ag/Ab Combo and an FDA-licensed HIV-1/2/O Antibody assay.

[b] 24 specimens were repeatedly reactive by both methods, of which 3 were positive by HIV-2 Western blot and 21 were positive by both HIV-1 and HIV-2 Western blot. These 21 specimens were further differentiated by an FDA-approved HIV-1/HIV-2 immunoassay; of these, 14 were HIV-1, 3 were HIV-2, and 4 were undifferentiated.

[c] One specimen was repeatedly reactive by both methods and was negative by HIV-1 Western blot and was confirmed positive by the HIV-1 p24 Antigen Assay and HIV-1 RNA PCR.

Of the 513 specimens from the HIV-2 endemic area, 89 (17.35%) were repeatedly reactive by the ARCHITECT HIV Ag/Ab Combo assay. Of the repeatedly reactive specimens, 83 were confirmed positive by HIV-1 Western blot, HIV-2 Western blot, HIV-1 p24 antigen assay, or HIV-1 RNA PCR. Of these, 1 was negative by HIV-1 and HIV-2 Western blot and positive by HIV-1 p24 antigen assay and HIV-1 RNA PCR.

A comparison of the ARCHITECT HIV Ag/Ab Combo results versus the FDA-licensed HIV-1/2/O Antibody assay results is summarized in Table 10. None of the specimens that were repeatedly reactive only on ARCHITECT HIV Ag/Ab Combo or only on the FDA-licensed Antibody assay were confirmed positive.

Table 10: ARCHITECT HIV Ag/Ab Combo and FDA-Licensed HIV-1/2/O Antibody Assay Results in Individuals at Increased Risk for Infection with HIV-2

ARCHITECT HIV Ag/Ab Combo	FDA-Licensed HIV-1/2/O Antibody Assay		
	Repeatedly Reactive	Nonreactive	Total
Repeatedly Reactive	86	3[a]	89
Nonreactive	1[b]	423	424

ARCHITECT HIV Ag/Ab Combo	FDA-Licensed HIV-1/2/O Antibody Assay		
	Repeatedly Reactive	Nonreactive	Total
Total	**87**	**426**	**513**

[a] One specimen was repeatedly reactive by HIV-2 EIA and indeterminate by HIV-2 Western blot, and two specimens were nonreactive by HIV-2 EIA. All 3 specimens were negative by HIV-1 Western blot, HIV-1 p24 Antigen Assay, and HIV-1 RNA PCR.

[b] This specimen was negative by HIV-1 Western blot, HIV-2 EIA, HIV-1 p24 Antigen Assay, and HIV-1 RNA PCR.

Pregnant Females

Four hundred fifty-three (453) specimens from a pregnant female US population were tested using the ARCHITECT HIV Ag/Ab Combo assay and an FDA-licensed HIV-1/2/O Antibody assay. The specimens included individuals at low risk for HIV infection (n=250, Table 1) and individuals at increased risk for HIV infection (n=203). In addition, 60 specimens from known HIV-1 antibody positive pregnant females (n=60) were tested using the ARCHITECT HIV Ag/Ab Combo assay.

Reactivity in Pregnant Females at Increased Risk for Infection with HIV

For the 203 specimens from pregnant females at increased risk for infection with HIV, the risk factors, if known, were documented and included: multiple sex partners during pregnancy, unprotected sex with an HIV-infected individual, unprotected sex with an HIV high-risk individual, unprotected sex with an individual diagnosed or treated for an STD, and history of sexually transmitted disease. The reactivity in this population is presented in Table 11. There were no specimens that were repeatedly reactive on the FDA-licensed Antibody assay and confirmed positive that were nonreactive on the ARCHITECT HIV Ag/Ab Combo assay.

Table 11: Reactivity of the ARCHITECT HIV Ag/Ab Combo Assay in Pregnant Females at Increased Risk for Infection with HIV

Specimen Category	Number of Specimens Tested	ARCHITECT HIV Ag/Ab Combo		FDA-Licensed HIV-1/2/O Antibody Assay	
		Number of Nonreactive Specimens (%)	Number of Repeatedly Reactive Specimens (%)	Number of Repeatedly Reactive Specimens (%)	Number of Confirmed Positive Specimens (%)
First Trimester	30	27 (90.00)	3 (10.00)	3 (10.00)	3 (10.00)
Second Trimester	36	34 (94.44)	2 (5.56)	3 (8.33)	2 (5.56)
Third Trimester	137	137 (100.00)	0 (0.00)	3 (2.19)	0 (0.00)
Total	**203**	**198 (97.54)**	**5 (2.46)**	**9 (4.43)**	**5 (2.46)**[a]

[a] Confirmed positive by HIV-1 Western blot.

Specificity

Specificity was determined using presumed negative specimens from 448 pregnant females (age range: 16 to 44 years) based on the FDA-licensed HIV-1/2/O antibody assay and supplemental testing. The pregnant females specimens were prospectively collected plasma specimens from pregnant females across all trimesters. Specificity of the ARCHITECT HIV Ag/Ab Combo assay in a pregnant female population in this study was 100.00% (448/448) with an exact 95% confidence interval of 99.18% to 100.00%.

Sensitivity

Sensitivity was estimated using a total of 65 specimens, which included 60 serum and plasma specimens from pregnant females known to be HIV-positive by HIV-1 Western blot (refer to Table 12) and 5 specimens from the increased risk for HIV population that were confirmed positive by HIV-1 Western blot (refer to Table 11). The sensitivity of the ARCHITECT HIV Ag/Ab Combo assay in a pregnant female population in this study was 100.00% (65/65) with an exact 95% confidence interval of 94.48% to 100.00%.

The reactivity for the 60 known HIV positive pregnant females is presented in Table 12.

Table 12: Reactivity of the ARCHITECT HIV Ag/Ab Combo Assay in Pregnant Females Known To Be Infected with HIV

Specimen Category	ARCHITECT HIV Ag/Ab Combo	Number of HIV-1

	Number of Specimens Tested	Number of Repeatedly Reactive Specimens (%)	Western Blot Confirmed Specimens (%)
First Trimester	22	22 (100.00)	22 (100.00)
Second Trimester	18	18 (100.00)	18 (100.00)
Third Trimester	20	20 (100.00)	20 (100.00)
Total	**60**	**60 (100.00)**	**60 (100.00)**

Pediatric Subjects

A total of 591 prospectively-collected specimens obtained from pediatric subjects were tested by the ARCHITECT HIV Ag/Ab Combo assay and the FDA-licensed HIV-1/2/O Antibody assay. Of the 591 pediatric subjects, 542 (age range 2 to 21 years) were from the United States and 49 (age range: 17 to 21 years) were from an HIV-2 endemic area (Côte d'Ivoire). The distribution of the results by age range and gender for the 591 pediatric subjects is presented in Table 13.

Table 13: Reactivity of the ARCHITECT HIV Ag/Ab Combo Assay in Pediatric Subjects at Low and Increased Risk for Infection with HIV

		ARCHITECT HIV Ag/Ab Combo		FDA-Licensed HIV-1/2/O Antibody Assay		
Age Range	Gender	Number of Nonreactive Specimens (%)	Number of Repeatedly Reactive Specimens (%)	Number of Nonreactive Specimens (%)	Number of Repeatedly Reactive Specimens (%)	Number of Confirmed Positive Specimens (%)
2 to 5 Years	Female	6 (100.00)	0 (0.00)	6 (100.00)	0 (0.00)	0 (0.00)
	Male	5 (100.00)	0 (0.00)	5 (100.00)	0 (0.00)	0 (0.00)
6 to 10 Years	Female	7 (100.00)	0 (0.00)	7 (100.00)	0 (0.00)	0 (0.00)
	Male	13 (100.00)	0 (0.00)	13 (100.00)	0 (0.00)	0 (0.00)
11 to 15 Years	Female	14 (100.00)	0 (0.00)	14 (100.00)	0 (0.00)	0 (0.00)
	Male	12 (100.00)	0 (0.00)	12 (100.00)	0 (0.00)	0 (0.00)
16 to	Female	358 (99.17)	3 (0.83)	356 (98.61)	5 (1.39)	2 (0.55)

Age Range	Gender	ARCHITECT HIV Ag/Ab Combo		FDA-Licensed HIV-1/2/O Antibody Assay		
		Number of Nonreactive Specimens (%)	Number of Repeatedly Reactive Specimens (%)	Number of Nonreactive Specimens (%)	Number of Repeatedly Reactive Specimens (%)	Number of Confirmed Positive Specimens (%)
21 Years	Male	172 (99.42)	1 (0.58)	172 (99.42)	1 (0.58)	1 (0.58)
Total		**587 (99.32)**	**4 (0.68)**	**585 (98.98)**	**6 (1.02)**	**3 (0.51)**[a]

[a] Includes individuals in the following categories: two (2) individuals at increased risk for HIV from an HIV-2 endemic area confirmed positive by HIV Western blot, and one (1) individual at low risk for HIV confirmed positive by HIV-1 Western blot.

Specificity

Specificity was determined using the 588 presumed negative specimens based on the FDA-licensed HIV-1/2/O Antibody assay and supplemental testing. The specificity of the ARCHITECT HIV Ag/Ab Combo assay in a pediatric population in this study was 99.83% (587/588) with an exact 95% confidence interval of 99.06% to 100.00%.

Sensitivity

Sensitivity was determined from 64 specimens, which included 61 specimens known to be HIV-1 positive by HIV-1 Western blot (Table 14), 1 specimen from the low risk for HIV population (refer to Table 1), and 2 specimens from the increased risk for HIV populations confirmed positive by supplemental testing (Table 15). The sensitivity of ARCHITECT HIV Ag/Ab Combo in a pediatric population in this study was 100.00% (64/64) with an exact 95% confidence interval of 94.40% to 100.00%.

The distribution of the results by age and gender for the 61 pediatric subjects known to be HIV-1 positive is presented in Table 14.

The reactivity in pediatric subjects at increased risk for infection with HIV-1 and HIV-2 is presented in Table 15. There were no specimens that were repeatedly reactive on the FDA-licensed Antibody assay and confirmed positive that were nonreactive on the ARCHITECT HIV Ag/Ab Combo assay.

Table 14: Reactivity of the ARCHITECT HIV Ag/Ab Combo Assay in Pediatric Subjects Known To Be Infected with HIV

Age Range	Gender	ARCHITECT HIV Ag/Ab Combo Number of Repeatedly Reactive Specimens (%)	Number of HIV-1 Western Blot Confirmed Specimens (%)
2 to 5 Years	Female	5 (8.20)	5 (100.00)
	Male	5 (8.20)	5 (100.00)
6 to 10 Years	Female	6 (9.84)	6 (100.00)
	Male	14 (22.95)	14 (100.00)
11 to 15 Years	Female	16 (26.23)	16 (100.00)
	Male	3 (4.92)	3 (100.00)
16 to 21 Years	Female	3 (4.92)	3 (100.00)
	Male	9 (14.75)	9 (100.00)
Total		**61 (100.00)**	**61 (100.00)**

Table 15: Reactivity of the ARCHITECT HIV Ag/Ab Combo Assay in Pediatric Subjects at Increased Risk for Infection with HIV

Specimen Category	Number Tested	ARCHITECT HIV Ag/Ab Combo Number of Repeatedly Reactive Specimens (%)	FDA-Licensed HIV-1/2/O Antibody Assay Number of Repeatedly Reactive Specimens (%)	Repeatedly Reactive Specimens (Number Reactive/Positive by Method) HIV-1 Western Blot (%)	HIV-2 Western Blot (%)	HIV-1 p24 Antigen (%)	HIV-1 RNA PCR (%)
Individuals at Increased Risk for HIV Infection - US Population	7	0 (0.00)	1 (14.29)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Individuals at Increased Risk for HIV Infection -	49	2 (4.08)	2 (4.08)	2[a] (4.08)	1[a] (2.04)	0 (0.00)	0 (0.00)

Specimen Category	Number Tested	ARCHITECT HIV Ag/Ab Combo	FDA-Licensed HIV-1/2/O Antibody Assay	Repeatedly Reactive Specimens (Number Reactive/Positive by Method)			
		Number of Repeatedly Reactive Specimens (%)		HIV-1 Western Blot (%)	HIV-2 Western Blot (%)	HIV-1 p24 Antigen (%)	HIV-1 RNA PCR (%)
HIV-2 Endemic Area							
Pregnant Females at Increased Risk for HIV Infection	38	0 (0.00)	1 (2.63)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Pediatric Population at Risk for HIV Infection	88	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)

[a] One specimen was positive by both HIV-1 and HIV-2 Western blot and further differentiated as HIV-1 by an FDA-approved HIV-1/HIV-2 immunoassay.

Summary of Results for Potentially Cross-Reacting Specimens

The ARCHITECT HIV Ag/Ab Combo assay was evaluated for potential cross-reactivity for specimens from individuals with medical conditions unrelated to HIV infection as summarized in Table 16. The specimens were tested using the ARCHITECT HIV Ag/Ab Combo assay and the FDA-licensed HIV-1/2/O Antibody Assay. The results for all 290 specimens were nonreactive with both assays.

Table 16: List of Potentially Cross-Reacting Specimens Tested

Category	N	Category	n	Category	n
Chlamydia	10	Hepatitis A Virus (anti-HAV positive)	10	Pregnancy Second Trimester	9
Common Cold	10	Hepatitis B Virus (anti-HBV positive)	10	Pregnancy Third Trimester	10
Crohn's Disease	10	Hepatitis C Virus (anti-HCV positive)	10	Rheumatoid Factor positive	10
Cytomegalovirus	9	Herpes Simplex	9	Rubella IgG	9

Category	N	Category	n	Category	n
(anti-CMV positive)		Virus (anti-HSV positive)			
Elevated IgG	7	Human Anti-Mouse Antibodies (HAMA) positive	10	Smallpox vaccine recipient	10
Elevated IgM	10	Human T-Lymphotropic Virus (HTLV)	6	Syphilis (positive serology)	10
Epstein-Barr Virus (anti-EBV positive)	10	IgM Monoclonal Gammopathy	9	Systemic Lupus Erythematosus (SLE & ANA)	14
Fungal Infections	10	Influenza vaccine recipient	10	Varicella Zoster Virus (anti-VZV positive)	10
Graves Disease	10	Multiparous Pregnancies	10	Viral Diarrheal Illness	10
Hemodialysis	8	Pregnancy First Trimester	10	Anti-*Escherichia coli* (anti-*E.coli* positive)	10

Interference

Potentially Interfering Substances

A study was performed to evaluate the susceptibility of the ARCHITECT HIV Ag/Ab Combo assay to elevated levels of potentially interfering substances based on guidance from CLSI EP07-A2.[39]

Each potential interferent was evaluated in both serum and plasma. Serum and plasma samples were prepared with no analyte and with 4 positive analyte types (anti-HIV-1, anti-HIV-1 group O, anti-HIV-2, and HIV-1 p24 antigen) at a level of approximately 3 S/CO (S/CO range: 2 to 4). These prepared specimens were supplemented with potential interferents as described below:

- Bilirubin test samples were prepared by adding bilirubin (conjugated and unconjugated) to a final concentration of ≥ 20 mg/dL to serum and plasma tubes containing no analyte (n = 43) and each positive analyte type (n = 177).
- Hemoglobin test samples were prepared by adding hemolysate containing hemoglobin to a final concentration of ≥ 500 mg/dL to serum and plasma tubes containing no analyte (n = 48) and each positive analyte type (n = 185).
- Triglycerides test samples were prepared by adding triglyceride stock solution to a final concentration of ≥ 1250 mg/dL to serum and plasma tubes containing no analyte (n = 47) and each positive analyte type (n = 191).

- Total Protein test samples were prepared by adding human serum protein to a final concentration of ≥ 12 g/dL to serum and plasma tubes containing no analyte (n = 49) and each positive analyte type (n = 174).

A mean S/CO change of no more than –20% for HIV-1 antigen, HIV-1 group M, HIV-1 group O, and HIV-2 antibody positive samples (S/CO range: 2 to 4) and a mean change of no more than 0.20 S/CO for nonreactive samples (S/CO range: < 1.00) were observed when serum and plasma specimens were spiked with elevated levels of bilirubin, hemoglobin, triglycerides, or total protein.

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