Intended for Use in the United States

INSTRUCTIONS FOR USE

aHIV

VITROS Immunodiagnostic Products
Anti-HIV 1+2 Reagent Pack

REF **680 1861**

Intended Use

For the *in vitro* qualitative detection of antibodies to Human Immunodeficiency Virus types 1 and/or 2 (anti-HIV-1 and anti-HIV-2) in human serum and plasma (heparin, EDTA or citrate) using the VITROS ECi/ECiQ Immunodiagnostic System.

The results of the VITROS Anti-HIV 1+2 assay, in conjunction with other serological evidence and clinical information, may be used as an aid in the diagnosis of infection with HIV-1 and/or HIV-2 in persons with signs or symptoms of, or at risk for, HIV infection.

WARNING: ***This assay has not been FDA cleared, licensed or approved for the screening of blood or plasma donors.***

Summary and Explanation of the Assay

Acquired Immunodeficiency Syndrome (AIDS) is caused by at least two types of Human Immunodeficiency Viruses designated HIV-1 and HIV-2. In addition, some HIV-1 strains have been isolated from AIDS patients in West Africa and designated as HIV-1 subtype O. Serological studies have shown that antibodies may develop to epitopes present in the peptides of the viral core and glycoprotein envelope. While antibodies to HIV-1 and HIV-2 core peptides demonstrate considerable cross reactivity, the antibodies generated by the glycoprotein envelope show less cross-reactivity.

The VITROS Anti-HIV 1+2 assay uses 4 recombinant antigens (HIV-1 Env 13, HIV-1 Env 10, HIV-1 p24, and HIV-2 Env AL) derived from HIV-1 core, HIV-1 envelope and HIV-2 envelope. HIV-1 Env 13, envelope SOD fusion protein, contains regions from both gp 120 and gp 41 regions. HIV-1 Env 10, envelope SOD fusion protein, contains a gp 41 region which extends beyond the C-terminus of Env 13. HIV-1 p24 is derived from full length core protein of HIV-1. HIV-2 Env AL, envelope SOD fusion protein, contains a region from gp 36 of HIV-2.

These antigens detect antibodies to HIV-1 and antibodies to HIV-2 in the same test. The use of these recombinant antigens improves assay specificity by avoiding non-specific reactions due to cross-reaction with human cell proteins, which are present in cell lysates.

The host organism for all four HIV recombinant antigens is S. cerevisiae (yeast).

Principles of the Procedure

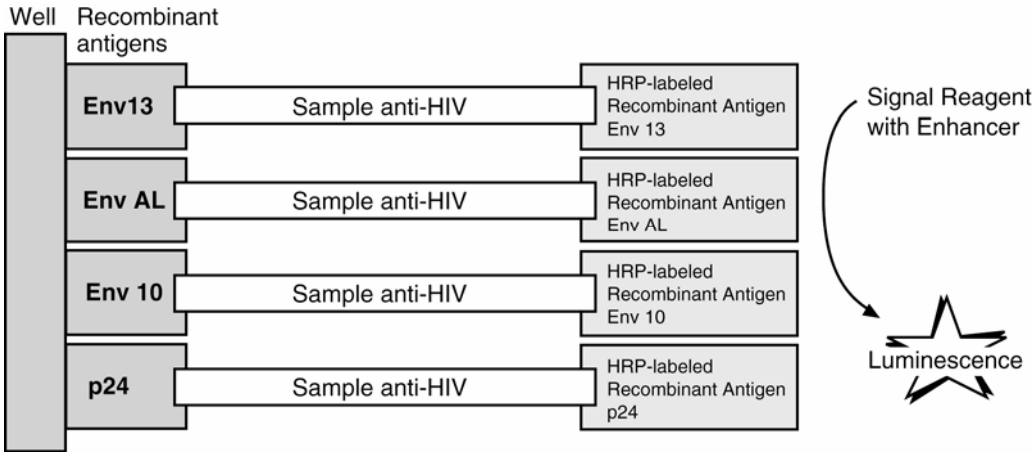
The VITROS Anti-HIV 1+2 assay is performed using the VITROS Immunodiagnostic Products Anti-HIV 1+2 Reagent Pack and the VITROS Immunodiagnostic Products Anti-HIV 1+2 Calibrator on the VITROS ECi/ECiQ Immunodiagnostic System.

An immunometric bridging technique is used; this involves a two-stage reaction. In the first stage HIV antibody present in the sample binds with HIV recombinant antigen coated on the wells. Unbound sample is removed by washing. In the second stage horseradish peroxidase (HRP)-labeled recombinant HIV antigens are added in the conjugate reagent. The conjugate binds specifically to any human anti-HIV-1 or anti-HIV-2 (IgG and IgM) captured on the well in the first stage. Unbound conjugate is removed by washing.

The bound HRP conjugate is measured by a luminescent reaction. [1] A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the VITROS ECi/ECiQ Immunodiagnostic System. The amount of HRP conjugate bound is indicative of the level of anti-HIV 1 and anti-HIV-2 present.

Assay Type	Assay Time and Temperature	
Immunometric assay	Incubation time: Time to first result: Temperature:	37 minutes 48 minutes 37 °C

Reaction Scheme



Warnings and Precautions

For *in vitro* Diagnostic Use Only.

WARNING: **Potentially Infectious Material**

Treat as if capable of transmitting infection.

Handling of samples and assay components, their use, storage, and solid and liquid waste disposal should be in accordance with the procedures defined by the appropriate national biohazard safety guideline or regulation (e.g. CLSI document M29). [2,3]

The* VITROS *Anti-HIV 1+2 Calibrator contains:

HIV antibody positive plasma obtained from donors who were tested individually and found to be negative for hepatitis B surface antigen (HBsAg), and for antibodies to hepatitis C virus (HCV), using FDA approved methods (enzyme immunoassays, EIA). The HIV antibody positive plasma has been treated in order to reduce the titer of potentially infectious virus. However, as no testing method can rule out the risk of potential infection, handle as if capable of transmitting infection.

HIV antibody negative plasma obtained from donors who were tested individually and found to be negative for hepatitis B surface antigen(HBsAg), and for antibodies to hepatitis C virus (HCV) and HIV, using FDA approved methods (enzyme immunoassays, EIA).

Care should be taken when handling material of human origin. All samples should be considered potentially infectious. No test method can offer complete assurance that hepatitis B virus, HCV, HIV 1+2 or other infectious agents are absent.

WARNING: **Contains Kathon**

The conjugate reagent in the* VITROS *Anti-HIV 1+2 Reagent Pack contains Kathon (1.0% w/w).

The assay reagent in the* VITROS *Anti-HIV 1+2 Reagent Pack contains Kathon (0.5% w/w).

The* VITROS *Anti-HIV 1+2 Calibrator contains Kathon (2.0% w/w).

R43: May cause sensitization by skin contact. S24: Avoid contact with skin. S37: Wear suitable gloves. [4]

Reagents

Reagent Pack Contents

One VITROS Anti-HIV 1+2 Reagent Pack, 100 tests (CAT No. 680 1861) contains:

- 100 coated wells (Human Immunodeficiency Virus Recombinant Antigens Env 13, Env AL, Env 10, p24 derived from yeast [S. cerevisiae]; coated at 0.36 µg/well).
- 6.2 mL Assay Reagent (buffer with anti-microbial agent [0.5% Kathon w/w]).
- 13.3 mL Conjugate Reagent (HRP labeled HIV -1[Env 13, Env 10, p24] and HIV- 2 [Env AL] recombinant antigens [1 to 3 µg/well] in buffered fetal calf serum with anti-microbial agent [1% Kathon w/w]).

Reagent Pack Handling

- The Reagent Pack is supplied ready for use.
- Reagent Packs do not need mixing.
- Avoid agitation, which may cause foaming or the formation of bubbles.

Reagent Pack Stability

When stored and handled as specified in the package labeling, the VITROS Anti-HIV 1+2 Reagent Pack is suitable for use until the expiration date printed on the outside of the carton.

Reagent Pack Storage and Preparation

- Store the unopened Reagent Pack refrigerated at 2–8 °C (36–46 °F). Do not freeze.
- Load Reagent Packs directly from refrigerated storage to minimize condensation.
- Use opened Reagent Packs within 8 weeks.
- Store opened Reagent Packs in the VITROS ECi/ECiQ Immunodiagnostic System Reagent Supply, or refrigerated at 2–8 °C (36–46 °F) in a sealed Reagent Pack storage box that contains dry desiccant.

Specimen Collection and Preparation

Patient Preparation

No special patient preparation is necessary.

Recommended Specimen Types

Serum or heparin, EDTA or citrated plasma.

Results from citrated plasma will be proportionately lower due to dilution by the anticoagulant.

Specimens Not Recommended

Turbidity in samples may affect assay results.

Special Precautions

Some sample collection devices have been reported to be detrimental to the integrity of certain analytes, and could interfere with some method technologies. [5] Because of the variety of sample collection devices available, it is not possible to issue a definitive statement on the performance of VITROS Immunodiagnostic Products when used with these devices. Each user should confirm that the chosen device is used according to the manufacturer's instructions and is compatible with this assay.

Specimen Collection and Preparation

- Collect specimens using standard procedures. [6]
- The VITROS Anti-HIV 1+2 assay uses 80 µL of sample for each determination.
- For details on minimum fill volume of sample cups or containers, refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide.
- Mix samples, calibrator, and controls by inversion and bring to 15–30 °C (59–86 °F) before use.
- Samples should be thoroughly separated from all cellular material. Failure to do so may lead to an erroneous result.

Handling and Storage Conditions

- Handle specimens in stoppered containers to avoid cross-contamination and evaporation. Use a separate disposable tip if samples are manually pipetted. Avoid splashing, forming an aerosol, or cross-contaminating sample tube stoppers.

- The amount of time samples are on board the system prior to analysis should be limited to avoid evaporation. This time should not exceed two hours. Refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide for further information.
- The Clinical and Laboratory Standards Institute (CLSI) provides the following recommendations for storing specimens: [7]
  - Store samples at 22 °C (72 °F) for no longer than 8 hours.
  - If the assay will not be completed within 8 hours, refrigerate samples at 2–8 °C (36–46 °F).
  - If the assay will not be completed within 48 hours, or for shipment, freeze samples at or below -20 °C (-4 °F).
  - Samples are not to be repeatedly frozen and thawed because this can cause analyte deterioration. Samples are to be thawed only once.

Assay Procedure

Materials Required But Not Provided (Sold Separately)

The following items are required to perform the VITROS Anti-HIV 1+2 assay:

- VITROS Immunodiagnostic System
- VITROS Anti-HIV 1+2 Calibrator, Calibrator Card, and Protocol Card
- VITROS Immunodiagnostic Products Signal Reagent
- VITROS Immunodiagnostic Products Universal Wash Reagent
- Quality control materials, such as VITROS Immunodiagnostic Products Anti-HIV 1+2 Controls
- VITROS Immunodiagnostic Products Reagent Pack Storage Box (optional) with desiccant

Operating Instructions

Refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide for complete instructions on the operation of your VITROS ECi/ECiQ Immunodiagnostic System.

Sample Dilution

WARNING: ***Automatic dilution is not available for this assay on the* VITROS *Immunodiagnostic System.***

Do not use manually diluted samples.

Calibration

Required Calibrator

VITROS Anti-HIV 1+2 Calibrator

Calibrator Preparation, Handling, and Storage

Refer to the Calibrator instructions for use for information on the use of the VITROS Anti-HIV 1+2 Calibrator.

Calibration Procedure

- Calibration must be performed using a Calibrator of the same lot number as the Reagent Pack.
- Refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide for detailed instructions on the calibration process.

When to Calibrate

- Calibrate when the lot of Reagent Pack and calibrator changes.
- Calibrate at least once every 28 days.

The VITROS Anti-HIV 1+2 assay may also need to be recalibrated:

- After specified service procedures have been performed (see the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide).
- If quality control results are consistently outside of the manufacturer's or your acceptable range.

Quality Control

Procedure Recommendations

- Choose control levels that check performance at clinically relevant points. The recommendation is to run a positive control close to the anti-HIV 1+2 decision point (the cutoff) and a negative control.
- To verify system performance, analyze control materials:
  - After calibration
  - At least once every 24 hours
  - After specified service procedures or maintenance to critical parts or subsystems that might influence performance of the assay (see the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide)
- Analyze quality control materials in the same manner as patient specimens.
- If control results fall outside the stated range or outside your established acceptable range, patient results should not be reported. Investigate and determine the cause for the unacceptable control results. When the condition is corrected, retest the controls and specimens.
- For more detailed information on quality control procedures, refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide.
- Refer to *Internal Quality Control Testing: Principles and Definitions* or other published guidelines for general quality control recommendations.[8]
- Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

Quality Control Material Selection

Choose control material that has a composition similar to or identical with the patient sample matrix being analyzed.[8]

VITROS Anti-HIV 1+2 Controls or similar material, are recommended for use with the VITROS ECi/ECiQ System. The performance of commercial (or non-commercial) control fluids, other than the VITROS Anti-HIV 1+2 Controls should be evaluated for compatibility with this assay before they are used for quality control.

Appropriate quality control value ranges must be established for all quality control materials used with the VITROS Anti-HIV 1+2 assay.

Quality Control Material Preparation and Storage

Refer to the manufacturer's product literature for preparation, storage, and stability information or establish preparation, storage and stability specifications for non-commercial material.

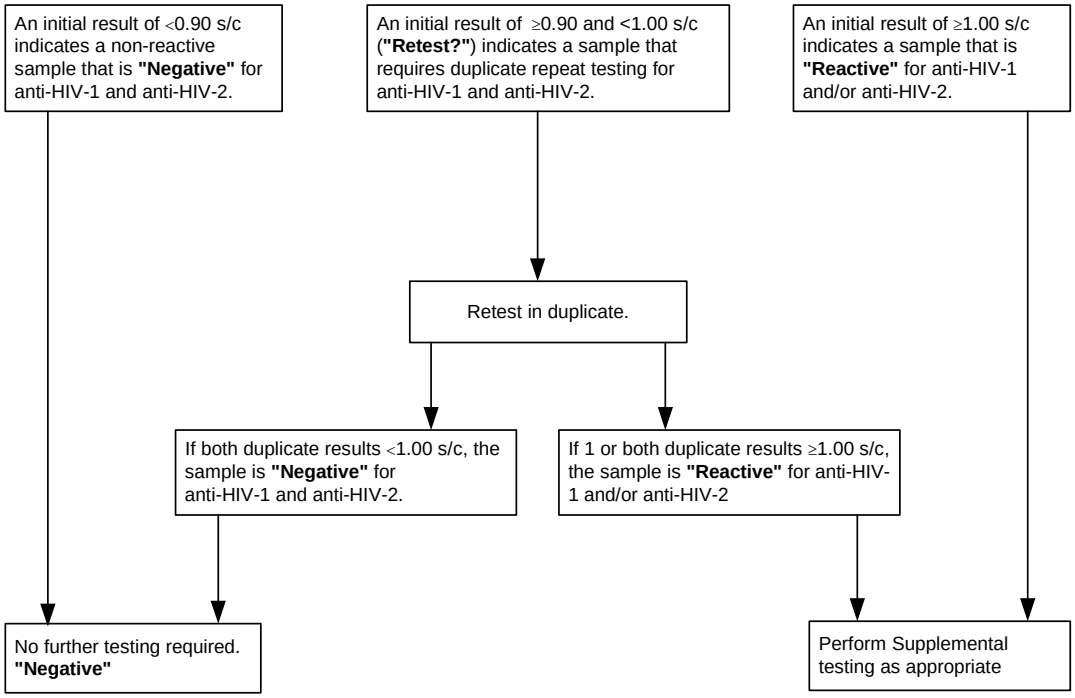
Interpretation of Results and Expected Results

Results are calculated as a normalized signal, relative to a cutoff value. During the calibration process, a lot-specific parameter, encoded on the lot calibration card, is used to determine a valid stored cutoff value for the VITROS ECi/ECiQ Immunodiagnostic System.

$$\text{Result} = \frac{\text{Signal for test sample}}{\text{Cutoff value}}$$

Caution:	**For this assay do not report aHIV results associated with VITROS ECi/ECiQ System codes CE (Calibration Expired), ED (Edited Results), EM (Expired Maintenance), IT (Incubator Temperature is outside Specifications), LT (Luminometer Temperature is outside specifications), M1 and/or M2 (Calibration data used for generating a calibration curve or patient results have changed from the default values), RC (Luminometer or Incubator reference readings are outside specifications), RE (Reagent Expired), and WT (Well wash temperature is outside specifications).**

Testing Algorithm



Interpretation of Results

The following table summarizes the interpretation of results obtained with the VITROS Anti-HIV 1+2 assay on the VITROS ECi/ECiQ Immunodiagnostic System.

VITROS Anti-HIV 1+2 Assay Result (s/c)	Conclusion from Testing Algorithm	Interpretation
<0.90	Negative	Specimen is negative for anti-HIV-1 and anti-HIV-2.
≥0.90 and <1.00	Retest in duplicate	Specimen is negative for anti-HIV-1 and anti-HIV-2 if both duplicate results are <1.00 s/c. Specimen is reactive for anti-HIV-1 and/or anti-HIV-2 if 1 or both duplicate results are ≥1.00 s/c.
≥1.00	Reactive	Specimen is reactive for anti-HIV-1 and/or anti-HIV-2.

Patient sample results will be displayed with a **"Negative"**, **"Retest?"** or "**Reactive**" label. An initial result labeled with **"Retest?"** indicates a sample that requires duplicate repeat testing. A duplicate result labeled with **"Retest?"** does not require further testing using the VITROS Anti-HIV 1+2 assay. Any result labeled with **"Reactive"** requires supplemental testing.

Result (s/c)	<0.90	≥0.90 and <1.00	≥1.00
Result Text	**Negative**	**Retest?**	**Reactive**

- If a specimen is reactive (result ≥1.00 s/c) the probability that HIV antibodies are present is high, especially in subjects at high risk for HIV infection. In most settings, it is appropriate to investigate reactive results by additional, more specific tests. Specimens found reactive by the VITROS Anti-HIV 1+2 assay and positive by additional, more specific tests are considered positive for antibodies to HIV-1 and/or HIV-2. Clinical correlation is indicated with appropriate counseling, medical intervention and possibly additional testing to decide whether a diagnosis of HIV infection is accurate.
- Interpretation of results from specimens found to be reactive by the VITROS Anti-HIV 1+2 assay and negative by additional, more specific tests is unclear. Further clarification may be obtained by testing another specimen obtained three to six months later.

- The magnitude of a VITROS Anti-HIV 1+2 assay result cannot be correlated to an endpoint titer.

Limitations of the Procedure

- The results from this or any other diagnostic kit should be used and interpreted only in the context of the overall clinical picture. A negative test result does not exclude the possibility of exposure to or infection with HIV. Levels of HIV antibodies may be undetectable in the early stages of infection.
- This test has not been validated for use with specimens from individuals less than 2 years of age.
- Samples with Total Protein > 9 g/dL may give falsely reactive results.
- Heterophilic antibodies in serum or plasma samples may cause interference in immunoassays. [9] These antibodies may be present in blood samples from individuals regularly exposed to animals or who have been treated with animal serum products. Results, which are inconsistent with clinical observations, indicate the need for additional testing.

Other Limitations

- A person who has antibodies to HIV-1 or HIV-2 is presumed to be infected with the virus, except that a person 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 decide whether a diagnosis of HIV infection is accurate.
- If your laboratory processes patient samples using a finite set of sample IDs (i.e. re-use of sample IDs over time), refer to the VITROS ECi/ECiQ Immunodiagnostic System Operator's Guide, Deleting Programs section regarding the steps that need to be followed to avoid the potential of a sample programming mismatch.

Performance Characteristics

Clinical Performance

A multi center study was conducted to establish the performance characteristics of the VITROS Anti-HIV 1+2 assay using samples obtained in the U.S. and internationally from individuals at low or high risk for HIV infection, or known to be HIV antibody positive. Statistical testing was performed to ensure that the distribution of VITROS Anti-HIV 1+2 s/c values was homogeneous across the three testing sites participating in the study.

The specificity of the VITROS Anti-HIV 1+2 assay was evaluated among individuals at low risk for HIV infection. The sensitivity of the VITROS Anti-HIV 1+2 assay was evaluated among individuals known to be HIV antibody positive, and by testing serially collected samples from individuals with HIV infection (seroconversion panels). Assay performance was further evaluated among individuals with signs or symptoms of HIV infection and among individuals belonging to groups recognized to be at risk for HIV infection due to lifestyle, behavior, occupation or known exposure event.

Results by Specimen Classification

Samples from subjects at high or low risk for HIV infection were tested with an FDA-licensed anti-HIV 1/2 assay, and with the VITROS Anti-HIV 1+2 assay at the three testing sites. The HIV antibody status (HIV Antibody Positive, HIV Antibody Negative or HIV antibody status Not Determined) of the individual subject was defined according to the following licensed and supplemental assay testing algorithm. In those instances where the licensed assay was negative but the VITROS Anti-HIV 1+2 assay was reactive, supplemental testing was performed to determine the HIV antibody status of the sample**.**

Licensed Anti-HIV 1/2 Assay Result	Supplemental Testing Result(s)	HIV Antibody Status
Negative	Not Applicable	HIV Antibody Negative
Reactive	Western blot (WB) Negative	HIV Antibody Negative*
Reactive	Western blot Positive	HIV Antibody Positive
Reactive	Western blot Indeterminate Indirect Immunofluorescence assay (IFA) Negative	HIV Antibody Negative*
Reactive	Western blot Indeterminate IFA Positive	HIV Antibody Positive
Reactive	Western blot Indeterminate IFA Indeterminate	HIV Antibody Status Not Determined **

* Samples from high risk subjects whose anti-HIV 1/2 assay results were discordant were tested with an HIV-2 EIA/IFA. The HIV antibody status remained "Negative" if the HIV-2 EIA was negative. If the HIV-2 EIA was repeatedly reactive and the HIV-2 IFA was negative or indeterminate, the HIV status was "Not Determined". The HIV status was "Positive" if the HIV-2 IFA was positive.

** These samples were tested with an HIV-2 EIA/IFA. HIV antibody status remained "Not Determined" if the HIV-2 EIA was negative, or if the HIV-2 EIA was repeatedly reactive but the HIV-2 IFA was negative or indeterminate. The HIV antibody status was "Positive" if the HIV-2 IFA was positive.

The specificity/negative percent agreement of the VITROS Anti-HIV 1+2 assay was calculated as the percentage of the combined HIV Antibody Negative and status Not Determined subjects that tested negative with the VITROS assay. The sensitivity/positive percent agreement of the VITROS Anti-HIV 1+2 assay was calculated as the percentage of HIV Antibody Positive subjects that tested reactive with the assay.

Specificity in Individuals at Low Risk for HIV Infection

Samples from 1444 subjects at low risk for HIV infection were tested with the VITROS Anti-HIV 1+2 and licensed anti-HIV 1/2 assays (with supplemental testing by licensed HIV-1 Western blot as required). These samples were obtained from pregnant women in the U.S. (N=297), pregnant women in the U.S. in the period around labor and delivery (N=49), from insurance applicants in the U.S. for whom HIV testing was required (N=999), and from pediatric subjects ages 2-17 years (N=99).

There were 99 unlinked samples from low risk pediatric subjects tested with the VITROS Anti-HIV 1+2 assay. The group was 49.5% male and 50.5% female and ranged in age from 2-17 years. None of the 99 samples was reactive with the VITROS Anti-HIV 1+2 assay. The distribution of VITROS Anit-HIV 1+2 assay negative results among the low risk pediatric subjects by age and gender is presented in the following table.

Demographics of seroreactivity in Low Risk Pediatric Population (N=99)

Age Range	Gender	VITROS Anti-HIV 1+2 Assay Results				Total
		Reactive		Negative		
		N	Percent	N	Percent	
2–4	Female	0	0.0	13	100.0	13
	Male	0	0.0	12	100.0	12
5–9	Female	0	0.0	11	100.0	11
	Male	0	0.0	14	100.0	14
10–14	Female	0	0.0	10	100.0	10
	Male	0	0.0	14	100.0	14
15–17	Female	0	0.0	16	100.0	16
	Male	0	0.0	9	100.0	9
Total		0	0.0	99	100.0	99

The results obtained from the 1444 low risk subjects are summarized in the following table.

VITROS Anti-HIV 1+2 and Licensed Anti-HIV 1/2 Assay Results in Low Risk Populations (N=1444)

Population Description	Number Tested	Licensed Anti-HIV 1/2 Assay			VITROS Anti-HIV 1+2 Assay		Total WB Positive
		NR	IR	RR (WB+)	NR	Reactive (WB+)	
Pregnancy (Low Risk–U.S.)	297	295	2	2 (0)	294	3 (1)	1
Labor & Delivery (Low Risk–U.S.)	49	49	0	0 (0)	48	1 (0)	0
Insurance Applicants (Low Risk–U.S.)	999	993	6	6 (2)	991	8 (5)	5
Pediatric (Low Risk–U.S.)	99	99	0	0 (0)	99	0 (0)	0
Total	1444	1436	8	8 (2)	1432	12 (6)	6

NR = non reactive (negative); IR = initially reactive; RR = repeatedly reactive; WB = licensed HIV-1 Western blot

Six of the 1444 low risk samples were reactive with the VITROS Anti-HIV 1+2 assay and positive on Western blot. Only two of those Western blot positive samples were repeatedly reactive with the licensed assay.

The performance of the VITROS Anti-HIV 1+2 assay compared with HIV antibody status in low risk populations is summarized in the following table:

Agreement of the VITROS Anti-HIV 1+2 Assay with HIV Antibody Status in Low Risk Populations (N=1444)

VITROS Anti-HIV 1+2 Assay Results	HIV Antibody Status			Total
	Positive	Negative	Not Determined*	
Reactive	6	5**	1	12
Negative	0	1432	0	1432
Total	6	1437	1	1444

*This sample remained HIV status "Not Determined" following VITROS, licensed and supplemental testing for anti-HIV-1 and anti-HIV-2 (EIA, WB, IFA). This sample was considered anti-HIV negative when calculating specificity.

**Three samples were initially reactive with the VITROS assay during the clinical study and were retested in duplicate: both duplicate results for the three samples were negative. All three were initially non reactive with the reference assay. HIV-1 Western blot results are not available for the three samples

The specificity of the VITROS Anti-HIV 1+2 assay was calculated as the percentage of the combined HIV antibody Negative and status "Not Determined" subjects that tested negative with the VITROS assay.

The specificity of the VITROS Anti-HIV 1+2 assay in the low risk populations was 99.58% (1432/1438 in this study with a 95% exact confidence interval (CI) of 99.09% to 99.85%) compared with 99.58% (1432/1438) for the licensed anti-HIV 1/2 assay. The six low risk samples that were HIV-1 Western blot positive (HIV antibody positive) were excluded from the specificity calculation.

Sensitivity in Individuals Positive for Antibodies to HIV-1 and HIV-2

Sensitivity in Individuals Positive for Antibodies to HIV-1

Samples from 1121 HIV-1 infected adults were tested with the VITROS Anti-HIV 1+2 assay. These subjects were enrolled in Florida (72.8%; N=816), California (16.3%; N=183), New Jersey (8.9%; N=100) and Texas (2.0%; N=22). Clinical and laboratory documentation of HIV-1 infection and HIV antibody positive status were obtained from medical records for each of the 1121 individuals. In addition, 40 unlinked residual samples from HIV-1 antibody positive pediatric subjects, ages 1-16 years of age, were also tested with the VITROS Anti-HIV 1+2 assay.

CD4+ counts were available for 1094 of the 1121 HIV-1 infected adults. As shown in the following table, the VITROS Anti-HIV 1+2 assay was reactive with all 1121 samples regardless of the subjects' CD4+ counts.

VITROS Anti-HIV 1+2 Assay Results Among U.S. HIV Infected Adults with Documented CD4+ Counts (N=1121)

CD4+ Count	N	VITROS Anti-HIV 1+2 Assay
		Reactive
< 200	149	149
200 - 499	429	429
> 499	516	516
Unknown	27	27

The VITROS Anti-HIV 1+2 assay results in the HIV-1 antibody positive populations are summarized in the following table:

VITROS Anti-HIV 1+2 Assay Results in Known HIV Antibody Positive Subjects (N=1161)

Population Description	VITROS Anti-HIV 1+2 Assay		
	N	NR	Reactive
Adult HIV-1 Positive (U.S.)	1121	0	1121
Pediatric HIV-1 Positive (U.S.)	40	0	40
Total	1161	0	1161

All 1161 samples were reactive with the VITROS Anti-HIV 1+2 assay. The sensitivity of the VITROS Anti-HIV 1+2 assay for U.S. subjects known to be positive for HIV-1 antibody was 100% (1161/1161; 95% CI = 99.68% to 100%) in this study.

Sensitivity in Individuals Positive for Antibodies to HIV-2

Sensitivity of the VITROS Anti-HIV 1+2 assay was also determined among 208 mono-infected HIV-2 antibody positive individuals from the Ivory Coast. Testing results are summarized in the following table:

VITROS Anti-HIV 1+2 Assay Results in Mono-Infected HIV-2 Antibody Positive Subjects (N=208)

Population Description	VITROS Anti-HIV 1+2 Assay		
	N	NR	Reactive
HIV-2 Positive (Ivory Coast)	208	0	208

All 208 anti-HIV-2 positive samples were reactive with the VITROS Anti-HIV 1+2 assay. The sensitivity of the VITROS Anti-HIV 1+2 assay in mono-infected HIV-2 antibody positive individuals was 100% (208/208; 95% CI = 98.24% to 100.0%) in this study.

Sensitivity in International Populations Known to be HIV Antibody Positive

Sensitivity of the VITROS Anti-HIV 1+2 assay was determined among 194 HIV antibody positive individuals from four geographic locations outside the U.S. Samples were obtained from archives in Africa (9.8%; N=19), Asia (53.1%; N=103), Europe (34.0%; N=66) and Latin America (3.1%; N=6). The VITROS Anti-HIV 1+2 assay results are presented in the following table:

VITROS Anti-HIV 1+2 Assay Results in International Populations Known to be HIV Antibody Positive (N=194)

Population Description	VITROS Anti-HIV 1+2 Assay		
	N	NR	Reactive
HIV Positive (Africa)	19	1*	18
HIV Positive (Asia)	103	0	103
HIV Positive (Europe)	66	0	66
HIV Positive (Latin America)	6	0	6
Total	194	1*	193

*This archived sample was obtained from a rural clinic in Keffi, Nigeria. The VITROS Anti-HIV 1+2 assay gave an initial s/c result of 0.53, and repeat results of 0.55 and 0.56. Singleton testing with the licensed assay gave an s/c result of 1.93. An HIV-1 Western blot showed bands of 1+ intensity at the p17, p24 and gp160 positions. The sample is unlinked to the donor's identity. No clinical information or follow-up sample is available.

One sample was non-reactive and 193 samples were reactive with the VITROS Anti-HIV 1+2 assay.

Reactivity in Populations at High Risk for HIV-1 Infection and HIV-2 Infection

Demographics of Seroreactivity Study in High Risk Populations

Among the 2175 high risk subjects from the U.S. participating in the VITROS Anti-HIV 1+2 assay clinical study, 1975 (90.8%) reported no current signs or symptoms of HIV infection. Of these 1975 asymptomatic individuals, 14.7% (291) were enrolled in Florida, 11.2% (222) were enrolled in New Jersey, 1.1% (21) were enrolled in Texas and 73.0% (1441) were enrolled in California. The group was Caucasian (28.2%), African American (18.1%) Hispanic (49.6%), and Asian (1.1%) with the remaining 3.0% represented by other ethnic groups. The group was 50.3% male and 49.7% female and ranged in age from 14 to 82 years. All were at risk for HIV infection due to lifestyle, behavior, occupation or known exposure event, or belonged to groups at risk for HIV infection. The VITROS Anti-HIV 1+2 assay was reactive in 2.2% (43/1975) of the individuals in this group. The percent VITROS Anti-HIV reactive results observed in the asymptomatic population in each collection area was 3.8% (11/291) in Florida, 0.0% (0/21) in Texas, 3.6% (8/222) in New Jersey, and 1.7% (24/1441) in California. The distribution of VITROS Anti-HIV 1+2 assay reactive and negative results among the high risk subjects without signs or symptoms of HIV infection by age and gender is presented in the following table.

Seroreactivity for the VITROS Anti-HIV 1+2 Assay in High Risk Subjects Without Signs or Symptoms of HIV Infection (N=1975)

Age Range	Gender	VITROS Anti-HIV 1+2 Assay Results				Total
		Reactive		Negative		
		N	Percent	N	Percent	
14–19	Female	0	0.0	28	100.0	28
	Male	0	0.0	25	100.0	25
20–29	Female	1	0.4	233	99.6	234
	Male	5	3.0	162	97.0	167
30–39	Female	1	0.4	284	99.6	285
	Male	5	2.0	249	98.0	254
40–49	Female	7	2.4	289	97.6	296
	Male	11	3.5	307	96.5	318
50–59	Female	2	1.7	116	98.3	118
	Male	11	5.6	184	94.4	195
60–69	Female	0	0.0	14	100.0	14
	Male	0	0.0	36	100.0	36
70–79	Female	0	0.0	1	100.0	1
	Male	0	0.0	3	100.0	3
80–82	Female	0	0.0	1	100.0	1
	Male	0	0.0	0	0.0	0
Total		43	2.2	1932	97.8	1975

Reactivity in Populations at High Risk for HIV-1 Infection

The performance of the VITROS Anti-HIV 1+2 assay was evaluated among 2175 individuals at high risk for HIV-1 infection prospectively enrolled in California (69.8%; N=1517), Florida (14.6; N=317), New Jersey (14.6%; N=317) and Texas (1.1%; N=24), and in 249 pregnant women at high risk for HIV infection enrolled in California (49.0%; N=122) and Florida (51.0%; N=127). Testing results are presented in the following table:

VITROS and Licensed Anti-HIV 1/2 Assay Results in U.S. High Risk Populations (N=2424)

Population Description	Number Tested	Licensed Anti-HIV 1/2 Assay			VITROS Anti-HIV 1+2 Assay		Total WB Positive
		NR	IR	RR (WB+)	NR	Reactive (WB+)	
High Risk (U.S.)	2175	2106	69	68 (53)	2116	59 (54)	54
Pregnancy (High Risk - U.S.)	249	244	5	4 (4)	243	6 (5)	5
Total	2424	2350	74	72 (57)	2359	65 (59)	59

The VITROS Anti-HIV 1+2 assay was reactive in 65 samples; 59 of those were Western blot positive. The licensed assay was repeatedly reactive in 57 (57/59; 99.61% detection). All of the 57 Western blot positives that were RR in the licensed test were reactive in the VITROS Anti-HIV 1+2 assay.

The performance of the VITROS Anti-HIV 1+2 assay compared with HIV antibody status in the U.S. high risk populations is summarized in the following table:

Agreement of the VITROS Anti-HIV 1+2 Assay with HIV Antibody Status in U.S. High Risk Populations (N=2424)

VITROS Anti-HIV 1+2 Assay Results	HIV Antibody Status			Total
	Positive	Negative	Not Determined*	
Reactive	59	5**	1	65
Negative	0	2359	0	2359
Total	59	2364	1	2424

*This sample remained HIV status "Not Determined" following VITROS, licensed and supplemental assay testing for anti-HIV-1 and anti-HIV-2 (EIA, WB, IFA). This sample was considered anti-HIV negative when calculating percent agreement.

** One sample was initially reactive with the VITROS assay during the clinical study and was retested in duplicate: both duplicate results were negative. The sample was initially non reactive with the reference assay. HIV-1 Western blot results are not available for the sample.

The positive percent agreement of the VITROS Anti-HIV 1+2 assay with HIV antibody status in U.S. high risk populations was 100% (59/59; 95% CI = 93.94% to 100%) compared with 96.61% (57/59) for the licensed assay. The negative percent agreement was 99.75% (2359/2365; 95% CI = 99.45% to 99.91%) in this study compared with 99.37% (2350/2365) for the licensed assay.

Reactivity in Populations at High Risk for HIV-2 Infection

The performance of the VITROS Anti-HIV 1+2 assay was evaluated among individuals at high risk for HIV-2 infection due to residence in an HIV-2 endemic area. The 488 subjects in this group were prospectively enrolled in the Ivory Coast. Testing results are presented in the following table:

VITROS and Licensed Anti-HIV 1/2 Assay Results in Individuals at High Risk for HIV-2 Infection (N=488)

Population Description	Number Tested	Licensed Anti-HIV 1/2 Assay			VITROS Anti-HIV 1+2 Assay		Total WB Positive
		NR	IR	RR (WB+)	NR	Reactive (WB+)	
High Risk (Ivory Coast)	488	453	35	33 (26)	457	31 (26)	26

The VITROS Anti-HIV 1+2 assay was reactive in 31 samples; 26 of these were HIV-1 Western blot positive. The licensed anti-HIV 1/2 assay was repeatedly reactive in 33 samples; 26 of these were HIV-1 Western blot positive. All of the 26 HIV-1 positives that were RR on the licensed test were reactive in the VITROS Anti-HIV 1+2 assay. The HIV-1 Western blot positive samples did not undergo further supplemental testing. Samples with discordant anti-HIV 1/2 EIA results that were negative or indeterminate by supplemental testing for antibodies to HIV-1 were tested for antibodies to HIV-2 (EIA and IFA). None were positive for anti-HIV-2.

The performance of the VITROS Anti-HIV 1+2 assay compared with HIV antibody status in the HIV-2 high risk population is summarized in the following table:

Agreement of the VITROS Anti-HIV 1+2 Assay with HIV Antibody Status in the HIV-2 High Risk Population (N=488)

VITROS Anti-HIV 1+2 Assay Results	HIV Antibody Status			Total
	Positive	Negative	Not Determined*	
Reactive	26	1	4	31
Negative	0	452	5	457
Total	26	453	9	488

*These samples remained HIV status "Not Determined" following VITROS, licensed and supplemental assay testing for anti-HIV-1 and anti-HIV-2 (EIA, WB, IFA). These samples were considered anti-HIV negative when calculating percent agreement.

The positive percent agreement of the VITROS Anti-HIV 1+2 assay with HIV antibody status in the HIV-2 high risk population was 100% (26/26; 95% CI = 86.77% to 100%) compared with 100% (26/26) for the licensed assay. The negative percent agreement was 98.92% (457/462; 95% CI = 97.49% to 99.65%) compared with 98.48% (455/462) for the licensed assay.

Positive and Negative Percent Agreement of the VITROS *Anti-HIV 1+2 Assay with HIV Antibody Status by Study Population*

The positive and/or negative percent agreement of the VITROS Anti-HIV 1+2 assay with HIV antibody status in the high risk, low risk and HIV antibody positive populations tested in this clinical study are summarized in the following table:

Positive and Negative Percent Agreement of the VITROS Anti-HIV 1+2 Assay with HIV Antibody Status by Study Population (N=5919)

Population	Positive Percent Agreement	95% Exact Confidence Intervals	Negative Percent Agreement	95% Exact Confidence Intervals
High Risk (U.S.)*	100% (59/59)	93.94%–100%	99.75% (2359/2365)	99.45%–99.91%
High Risk (Ivory Coast)	100% (26/26)	86.77%–100%	98.92% (457/462)	97.49%–99.65%
HIV Positive (U.S.)**	100% (1161/1161)	99.68%–100%		
HIV Positive (International)	99.48% (193/194)	97.16%–99.99%		
HIV-2 Positive (Ivory Coast)	100% (208/208)	98.24%–100%		
Low Risk (U.S.)***	100% (6/6)	N/A****	99.58% (1432/1438)	99.09%–99.85%

* Adult (N=2175); pregnant (N=249).

** Adult (N=1121); pediatric (N=40).

*** Pregnant (N=297); labor and delivery (N=49); insurance applicants (N=999); pediatric (N=99).

**** N/A=Not applicable. Confidence intervals calculated on small numbers are not meaningful.

Seroconversion Panels

Twenty commercially available seroconversion panels were tested. Results for the twenty panels are summarized in the following table. The table presents the days elapsed from the date of the initial bleed to the last negative sample and first reactive sample. Data are presented for both assays for each of the seroconversion panels.

Days to Evidence of HIV Infection

	Licensed Anti-HIV 1/2 Assay		VITROS Anti-HIV 1+2 Assay		Difference in Days to Anti-HIV Reactive Result
Panel ID	**–***	**+ ****	**–*****	**+ ********	**Licensed Assay minus VITROS Anti-HIV 1+2 Assay**
PRB904	49	92	49	92	0
PRB910	14	26	14	26	0
PRB916	15	30	15	30	0
PRB923	37	47	37	47	0
PRB924	26	33	26	33	0
PRB925	22	44	22	44	0
PRB926	9	27	9	27	0
PRB927	0	28	28	33	-5
PRB929	21	25	18	21	4
PRB931	15	28	15	28	0
PRB933	0	21	0	21	0
PRB934	0	7	0	0	7
PRB935	28	43	28	43	0
PRB940	7	11	0	7	4
PRB941	9	18	9	18	0
PRB944	9	14	9	14	0
PRB945	7	13	7	13	0
PRB947	0	9	0	9	0
PRB952	14	17	10	14	3
PRB959	7	9	0	7	2

* Post bleed day of last non-reactive result, usually denotes previous bleed from first repeatedly reactive result.

** Post bleed day of first repeatedly reactive result.

*** Post bleed day of last non-reactive result, usually denotes previous bleed from first reactive result.

**** Post bleed day of first reactive result.

The VITROS Anti-HIV 1+2 and licensed anti-HIV 1/2 assays were in agreement for 14 of the 20 panels. The VITROS Anti-HIV 1+2 assay became reactive one bleed (from two to seven days) earlier for five of the twenty panels. The licensed assay became repeatedly reactive one bleed (five days) earlier for the final panel.

Genotype Detection

Genotype detection was assessed using the Boston Biomedica, Inc. Worldwide HIV Performance Panel. This panel consists of 25 naturally occurring plasma specimens originating from diverse geographic locations. Twenty three of these specimens have been characterized to be anti-HIV reactive, while two are anti-HIV nonreactive. The reactive specimens represent HIV Group M (subtypes A, B, C, D, E, F, and G) Group O, and HIV-2 genotypes. All 23 of the anti-HIV reactive panel members were also reactive in the VITROS anti-HIV 1+2 assay, while the two anti-HIV nonreactive panel members were negative in the VITROS Anti-HIV 1+2 assay. Two lots of VITROS Anti-HIV 1+2 Reagent Packs and Calibrators were included in this study.

BBI Worldwide HIV Performance Panel Test Results with VITROS Anti-HIV 1+2 Assay

Panel ID Number	VITROS Anti-HIV 1+2 Assay		
	Genotype	Result (s/c)	Classification
WWRB302(M)-01	O	9.45	Reactive
WWRB302(M)-02	A	55.3	Reactive
WWRB302(M)-03	G	85.3	Reactive
WWRB302(M)-04	G	56.4	Reactive
WWRB302(M)-05	A	54.6	Reactive
WWRB302(M)-06	G	65.0	Reactive
WWRB302(M)-08	G	60.0	Reactive
WWRB302(M)-09	A	82.6	Reactive
WWRB302(M)-10	NEG	0.14	Negative
WWRB302(M)-11	HIV-2*	26.3	Reactive
WWRB302(M)-12	C	89.4	Reactive
WWRB302(M)-14	D	61.2	Reactive
WWRB302(M)-15	D	59.2	Reactive
WWRB302(M)-16	D	64.1	Reactive
WWRB302(M)-17	D	73.0	Reactive
WWRB302(M)-19	C	55.2	Reactive
WWRB302(M)-21	B'	81.4	Reactive
WWRB302(M)-22	E	72.7	Reactive
WWRB302(M)-24	E	78.4	Reactive
WWRB302(M)-25	HIV-2*	40.6	Reactive
WWRB302(M)-26	B	74.1	Reactive
WWRB302(M)-27	B/D	54.1	Reactive
WWRB302(M)-28	F	79.6	Reactive
WWRB302(M)-29	B	86.7	Reactive
WWRB302(M)-30	NEG	0.10	Negative

* The HIV-2 status of these specimens was determined by serological testing.

Detection of HIV-1 Group O Specimens

Thirteen confirmed HIV 1 Group O antibody positive samples were tested with the VITROS Anti-HIV 1+2 assay. All 13 samples gave a reactive result. All samples were confirmed as HIV O reactive using Western Blots developed at the Institut Alfred Fournier. The results are shown in the following table.

HIV-1 Group O Sample ID	VITROS Anti-HIV 1+2 Assay Result (s/c)
R407/937	25.4
R407/939	23.3
R407/941	5.85
R407/942	27.1
R407/943	10.1
R407/946	2.73
R407/947	4.08
R407/948	11.1
R407/949	26.1
R407/950	5.18
R407/951	8.69
R407/952	19.0
R407/953	36.6

One additional sample from the Worldwide HIV Performance Panel obtained from Boston Biomedica, Inc. (sample WWRB302(M)-01) was also tested with the VITROS Anti-HIV 1+2 assay, and gave a reactive s/c result of 9.45. Thus, all 14 HIV-1 Group O samples gave reactive results with the VITROS Anti-HIV 1+2 assay.

Potentially Cross-Reacting Sub-groups

A total of 236 patient samples from the following 14 potentially cross-reacting sub-groups were tested in the VITROS anti-HIV 1+2 assay: HCV infection, HBV infection, HTLV I/II antibody positive, EBV infection, Influenza vaccine recipients, multiply transfused patients, multiparous females, dialysis patients, hemophilia patients, autoimmune disease patients, high rheumatoid factor, yeast (Candida) reactive, SOD reactive samples, and cord blood samples from neonates. An additional 15 samples from patients who had not received the Influenza vaccine were tested as a control for the Influenza vaccine recipients group. In all, 251 patient samples were tested for this study.

In these 251 samples tested, 3 were reactive in the VITROS Anti-HIV 1+2 assay. In the 14 clinical categories, 1 sample from the hemophilia group was found to give a reactive result in the VITROS Anti-HIV 1+2 assay yielding results just above the cutoff. This sample yielded negative results with an FDA licensed anti-HIV 1/2 assay. The second reactive sample was from the pre-Influenza vaccine group, this sample was also reactive with an FDA licensed anti-HIV 1/2 assay. A third sample in the SOD reactive subgroup tested reactive on initial determination and negative upon repeat testing.

Summary of VITROS Anti-HIV 1+2 Assay Results with Potentially Cross-Reacting Specimens

Sample Category	No. Samples Tested	No. Negative	No. Reactive	No. Confirmed Positive
HCV Infection	16	16	0	0
HTLV I/II Positive	16	16	0	0
EBV Infection	15	15	0	0
Multiparous Females	16	16	0	0
Pre-Influenza Vaccine	15	14	1	0*
Post-Influenza Vaccine	15	15	0	0
Rheumatoid Factor	16	16	0	0
Autoimmune Disease	16	16	0	0
Multiply Transfused Patients	16	16	0	0
HBV Infection	16	16	0	0
Hemophilia	16	15	1	0
Dialysis	16	16	0	0
Yeast Reactive	20	20	0	0
SOD Reactive	22	21	1	0
Cord Blood (Neonates)	20	20	0	0

*Pre-Influenza specimen was tested and found reactive with an FDA licensed anti-HIV 1/2 assay. There was insufficient sample to perform a Western blot.

Potentially Cross-Reacting Sub-Groups - Microbiological Studies

The potential for bacterial contamination to affect the performance of the VITROS Anti-HIV 1+2 assay was evaluated further by testing samples spiked with *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. The samples were tested with and without a spike of anti-HIV 1+2.

Of the samples that were tested none of the anti-HIV 1+2 unspiked (negative) samples were found to be false reactive and none of the anti-HIV 1+2 spiked samples were observed to be false negative in the VITROS Anti-HIV 1+2 assay.

Potentially Interfering Substances

The potentially interfering effects of hemoglobin, bilirubin and triolein were evaluated using samples from 30 patients. The results demonstrate that hemoglobin (up to 500 mg/dL), bilirubin (up to 20 mg/dL) and triolein (up to 3000 mg/dL) cause no misclassification of results. Samples spiked with anti-HIV-1 and anti-HIV-2 reactive plasma were tested near the cutoff (cutoff s/c=1.00) and were observed to remain reactive at all levels tested with each potential interferent. Similarly, no interference was observed in samples not spiked with anti-HIV-1 and anti-HIV-2 reactive plasma, with results remaining below 1.00 s/c.

HIV-1			Mean Result at 0 Interferent Level		Mean Result at Maximum Interferent Level	
Test Substance	**Sample**	**Maximum Level Tested**	**s/c**	**Classification**	**s/c**	**Classification**
Hemoglobin	HIV-1 Spiked sample	500 mg/dL	1.11	Reactive	1.16	Reactive
	Negative sample	500 mg/dL	0.06	Negative	0.09	Negative
Bilirubin	HIV-1 Spiked sample	20 mg/dL	1.53	Reactive	1.51	Reactive
	Negative sample	20 mg/dL	0.08	Negative	0.08	Negative
Triolein	HIV-1 Spiked sample	3000 mg/dL	1.34	Reactive	1.36	Reactive
	Negative sample	3000 mg/dL	0.06	Negative	0.06	Negative

HIV-2			Mean Result at 0 Interferent Level		Mean Result at Maximum Interferent Level	
Test Substance	**Sample**	**Maximum Level Tested**	**s/c**	**Result**	**s/c**	**Result**
Hemoglobin	HIV-2 Spiked sample	500 mg/dL	1.21	Reactive	1.29	Reactive
	Negative sample	500 mg/dL	0.06	Negative	0.09	Negative
Bilirubin	HIV-2 Spiked sample	20 mg/dL	1.39	Reactive	1.40	Reactive
	Negative sample	20 mg/dL	0.17	Negative	0.18	Negative
Triolein	HIV-2 Spiked sample	3000 mg/dL	1.55	Reactive	1.55	Reactive
	Negative sample	3000 mg/dL	0.06	Negative	0.06	Negative

A total of 60 samples was tested from the following subsets: patients with Cholesterol <200 mg/dL, patients with Cholesterol >300 mg/dL, patients with Total Protein between 6 and 8 g/dL, patients with Total Protein >9 g/dL, normal patient samples (assumed normal IgG concentration) and patient samples spiked with Human IgG to achieve a concentration of >2620 mg/dL. Samples for Cholesterol and Total Protein testing were naturally occurring. Samples for IgG testing were normal patient samples that were spiked with a purified IgG preparation to achieve a level above the normal range.

All samples in each subset were spiked with anti-HIV-1 or anti-HIV-2 reactive plasma to evaluate performance with positive samples. All samples in each subset were also tested after being spiked with negative plasma to evaluate performance with negative samples.

All samples spiked with anti-HIV-1 or anti-HIV-2 reactive plasma yielded reactive results. Among samples spiked with negative plasma 47 of 60 yielded negative results. A total of 13 samples, all in the high total protein group, yielded reactive results. Twelve of 13 reactive samples also tested reactive with an FDA licensed method.

Patient samples spiked with cholesterol up to 415 mg/dL or IgG up to 2620 mg/dL do not interfere with the clinical interpretation of results.

Sample Category	No. Samples Tested	No. Negative	VITROS Anti-HIV 1+2 Assay Reactive	No. Reactive in FDA Licensed Assay
Cholesterol <200 mg/dL	10	10	0	0
Cholesterol >300 mg/dL*	10	10	0	0
Total Protein between 6 and 8 g/dL	10	10	0	0
Total Protein >9 g/dL	20	7	13	12
Serum spiked with Human IgG**	10	10	0	0

*Maximum cholesterol level in the tested sample was 415 mg/dL.

**Maximum IgG concentration was 2620 mg/dL.

A total of 22 additional samples were tested from patients with Total Protein >9 g/dL. Samples were tested to determine if the presence of high total protein would cause negative samples to yield reactive assay results.

A total of 21 of the 22 samples yielded negative results with the VITROS Anti-HIV 1+2 assay. A single sample yielded reactive results with the VITROS Anti-HIV 1+2 assay. This sample was determined to be reactive in an FDA licensed assay and confirmed HIV antibody positive by Western blot.

Patient samples containing protein >9 g/dL do not consistently interfere with the clinical interpretation of results.

Summary of VITROS anti-HIV 1+2 Data from Potentially Interfering Sample Conditions

Sample Category	No. Samples Tested	No. Negative	VITROS Anti-HIV 1+2 Assay Reactive	No. Reactive in FDA Licensed Assay	No. Confirmed HIV Antibody Positive in Western Blot
Total Protein >9 g/dL	22	21	1	1	1

Precision

Precision was evaluated on a different VITROS ECi/ECiQ Immunodiagnostic System at three external sites, using one reagent pack and calibrator kit lot. At least two replicates each of a four member panel were assayed on a single occasion per day on 20 different days. The data shown in the table were rounded following all calculations.

Clinical Site	Mean VITROS Anti-HIV 1+2 Assay Results (s/c)		Repeatability*		Between Day**		Total***		No. of Obs.	No. of Days
			S.D.	C.V.(%)	S.D.	C.V.(%)	S.D.	C.V.(%)		
Site 1	0.07	Negative	0.007	9.9	0.005	6.9	0.008	12.1	40	20
	6.06	HIV-1	0.071	1.2	0.172	2.8	0.186	3.1	40	20
	4.12	HIV-2	0.046	1.1	0.163	3.9	0.169	4.1	40	20
	1.32	HIV-1	0.023	1.8	0.046	3.5	0.052	4.0	40	20
Site 2	0.08	Negative	0.006	7.6	0.003	4.3	0.007	8.7	40	20
	6.66	HIV-1	0.169	2.5	0.163	2.5	0.235	3.5	40	20
	4.39	HIV-2	0.060	1.4	0.100	2.3	0.117	2.7	40	20
	1.39	HIV-1	0.045	3.2	0.035	2.5	0.057	4.1	40	20
Site 3	0.07	Negative	0.003	4.4	0.004	5.6	0.005	7.1	40	20
	6.22	HIV-1	0.085	1.4	0.131	2.1	0.156	2.5	40	20
	4.42	HIV-2	0.033	0.7	0.158	3.6	0.162	3.7	40	20
	1.34	HIV-1	0.028	2.1	0.034	2.5	0.044	3.3	40	20

* Repeatability: Variability of the assay performance from replicate to replicate.

** Between Day: Variability of the assay performance from day to day.

*** Total: Variability of the assay combining the effects of repeatability and between day.

Precision was further evaluated incorporating between site and between lot variation. The study was performed at three external sites using three reagent lots. At least three replicates each of a four member panel were assayed on a single occasion per day on six different days. The between site, between lot, and total precision estimates CV (%) were derived from a variance component analysis. The data shown in the table were rounded following all calculations.

Mean VITROS Anti-HIV 1+2 Assay Results (s/c)		Between Site*		Between Lot**		Total***		No. of Obs.
		S.D.	C.V. (%)	S.D.	C.V. (%)	S.D.	C.V. (%)	
0.10	Negative	0.004	3.9	0.031	30.0	0.034	32.0	162
1.09	HIV-2	0.000	0.0	0.152	14.0	0.164	15.0	162
1.34	HIV-1	0.000	0.0	0.017	1.3	0.070	5.2	162
3.76	HIV-1	0.049	1.3	0.210	5.6	0.275	7.3	162

* Between Site: Variability of the assay performance from site to site.

** Between Lot: Variability of the assay performance from lot to lot calculated using data across all sites.

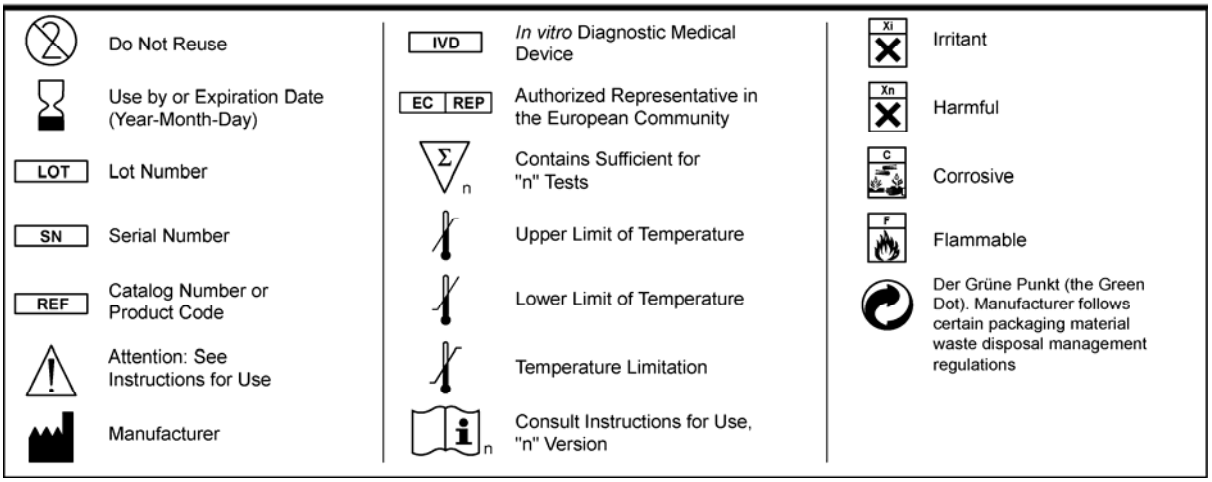
*** Total: Variability of the assay incorporating factors of site, lot and day.

References

1. Summers M et al. Luminogenic Reagent Using Chloro 4-Hydroxy Acetanilide to Enhance Peroxidase/Luminol Chemiluminscence, Clin Chem 41. S73 (1995).
2. CDC-NIH. Biosafety in Microbiological and Biomedical Laboratories – 3rd Edition. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C., 1993.
3. CLSI. *Protection of laboratory Workers from Occupationally Acquired Infections; Approved Guideline- Third Edition*. CLSI Document M29-A3 (ISBN 1-56238-567-4). CLSI, 490 West Valley Rd. Suite 1400, Wayne, PA 19087-1898 USA, 2005.
4. European 'Dangerous Preparations Directive (1999/45/EC)'.
5. Calam RR. Specimen Processing Separator Gels: An Update, J Clin Immunoassay 11: 86-90 (1988).
6. NCCLS. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture – Fifth Edition; Approved Standard. NCCLS document H3- A5 (ISBN 1-56238-515-1). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, 2003.
7. CLSI. Procedures for the Handling and Processing of Blood Specimens; Approved Guideline – Third Edition. CLSI document H18-A3 (ISBN 1-56238-555-0). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087, 1999.
8. NCCLS. Statistical Quality Control for Quantitative Measurements: Principles and Definitions: Approved Guideline-Second Edition. NCCLS document C24-A2 (ISBN 1-56238-371-X), NCCLS, 940 West Valley Road, Suite 1400. Wayne, Pennsylvania 19087, 1999.
9. Levinson SS. The Nature of Heterophilic Antibodies and Their Role in Immunoassay Interference , J Clin Immunoassay 15: 108-115 (1992).

Glossary of Symbols

The following symbols may have been used in the labeling of this product.



Revision History

Date of Revision:	Version:	Description:
2008-03-26	DRAFT Version 1.0	• Spelled out WB as Western blot • Changed instances of Western Blot to Western blot • Changed instances of compared to to compared with • Moved table from p. 11 to p. 8 and modified text as directed • Merged cord blood data with cross-reacting subgroups table
2008-03-24	DRAFT Version 1.0	• Requested edits.See yellow highlight text for changes or additions. See change bars for location of deletions. • Page 1, Summary and Explanation – 1st paragraph, last sentence:add hyphen to cross reactivity • Page 3, Reagents – Capitalize Assay Reagent, Conjugate Reagent, Reagent Packs, and Reagent Supply • Page 3, Handling and Storage Conditions- add hyphen to cross contaminating • Page 4, Sample Dilution – Remove "Anti-HIV 1+2 results (s/c) ≥1.00 should be reported as Reactive." Warning statement edited to "Automatic dilution is not available for this assay on the VITROS Immunodiagnostic System." • Page 4, Calibration – capitalize Calibrator , Reagent Pack, last sentence in When to Calibrate should be a bullet. • Page 5, Interpretation of Results and Expected Results, "To paragraph before table add" A duplicate result labeled with "Retest?" does not require further testing using the VITROS Anti-HIV 1+2 assay. A result labeled with "Reactive" requires supplemental testing." Move section to page 6 after Interpretation of Results table • Page 6 Testing Algorithm "If 1 or both" reversed in boxes third level down. In table 2nd row, Interpretation column same change was made. • Page 6, Interpretation of Results-first sentence remove "upon completion of all testing steps required in the testing algorithm." Remove 4th paragraph beginning "Results obtained with the VITROS Anti-HIV 1+2 assay may……." • Page 6, 7,Limitations of Procedure add 2nd bullet "This test has not been validated for use with specimens from individuals less than 2 years of age." Add third bullet "Samples with Total Protein . 9 g/dL…" • Page 7 Other Limitations Add 1st bullet "A person who has antibodies…" • Page 7, Move Expected Results to top of page 11 and change title to Demographics of Seroreactivity Study in High Risk Populations. • Page 7, Remove last bullet of "Limitations of the Procedure" • Page 8, Performance Characteristics table, 1st column title added "Licensed" • Page 9 Specificity in Individuals at Low Risk for HIV Infections, 1st sentence after table edited. 5th paragraph added "in this study" • Page 10, "subjects" changed to"individuals" 5 places, add "in this study" 2 places, add 95% to CI in 2 places • Page 11 Reactivity in Populations at High Risk for HIV-1 Infections, 2nd paragraph, add "reactive in 65 samples; 59 of those were WB positive" and "All of the 57 WB positives that were RR in the licensed test were reactive in the VITROS Anti-HIV 1+2 Assay." 3rd paragraph add 95% 2 places for CI and "in this study" • Page 12, 1str paragraph added "was reactive in 31 samples;

		26 of these were HIV-1 WB positive" and "was repeatedly reactive in 33 samples; 26 of these were HIV-1 WB positive. All of the 26 HIV-1 positives that were RR on the licensed test were reactive in the VITROS Anti-HIV 1+2 Assay." 3rd paragraph added 95% 2 places. • Page 13, 1st paragraph, last sentence changed "is" to "are" • Page 15, Potentially Cross-Reacting Sub-groups change "HTLV 1+2" to "HTLV I+II" and change FDA "approved method" to FDA "llicensed anti-HIV 1/2 assay" • Page 16 change "HTLV 1+2" to "HTLV I/II" in table, last row, 3rd column change 22 to 21 • Page 16 Potentially Cross-Reacting Sub-groups-Microbiological Studies, beginning of 1st sentence changed from "The specificity …" to "The potential for bacterial conramination to affect the performance……..". Change "an additional spike" to "a spike" • Page 17, last sentence change "approved" to "licensed" • Page 18, 3rd paragraph changed "FDA approved" to "FDA licensed"
2008-03-12	DRAFT Version 1.0	• Quality Control, Procedure Recommendations: Change second sentence in 1st bullet from "The recommendation is to run a negative control and a positive control close to the anti-HIV 1+2 decision point (signal/cutoff [s/c] ≥1.00)." to "The recommendation is to run a positive control close to the anti-HIV 1+2 decision point (the cutoff) and a negative control." Also removed the following last sentence in first bullet:" The VITROS anti-HIV 1+2 negative control is targeted to yield a result ≤0.2 s/c, the VITROS anti-HIV 1+2 positive controls are targeted to yield a result of approximately 6.0 s/c."
2008-03-06	DRAFT Version 1.0	• Updated Glossary of Symbols
2008-XX-XX	1.0	• Initial release of Instructions for Use

When this Instructions For Use is replaced, sign and date below and retain as specified by local regulations or laboratory policies, as appropriate.

_____ _____

Signature Obsolete Date

Conditions of supply: all supplies are made subject to the standard terms and conditions of Ortho Clinical Diagnostics or its distributors. Copies of these are available on request.

Co-developed with

CHIRON

Novartis Vaccines and Diagnostics, Inc.
Emeryville, CA 94608-2916

Ortho-Clinical Diagnostics
a Johnson & Johnson company

Ortho-Clinical Diagnostics
Johnson & Johnson
50-100 Holmers Farm Way
High Wycombe
Buckinghamshire
HP12 4DP
United Kingdom