

# Multi-Center Outcomes-Based Clinical Evaluation of the VITROS® Immunodiagnostic Products Anti-HIV 1+2 Assay (VITROS Anti-HIV 1+2 Assay) in Subjects at High Risk, Low Risk or Positive for Infection with Human Immunodeficiency Virus Types 1 and/or 2 (HIV-1 and/or HIV-2)

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## Introduction

This multi-center clinical study was conducted to establish the performance characteristics of the VITROS Anti-HIV 1+2 assay versus an FDA-licensed anti-HIV 1/2 reference assay. The VITROS Anti-HIV 1+2 assay is an immunometric assay 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 assay is intended, in conjunction with other serological evidence and clinical information, 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.

## Materials and Methods

### PRINCIPLES OF THE PROCEDURE

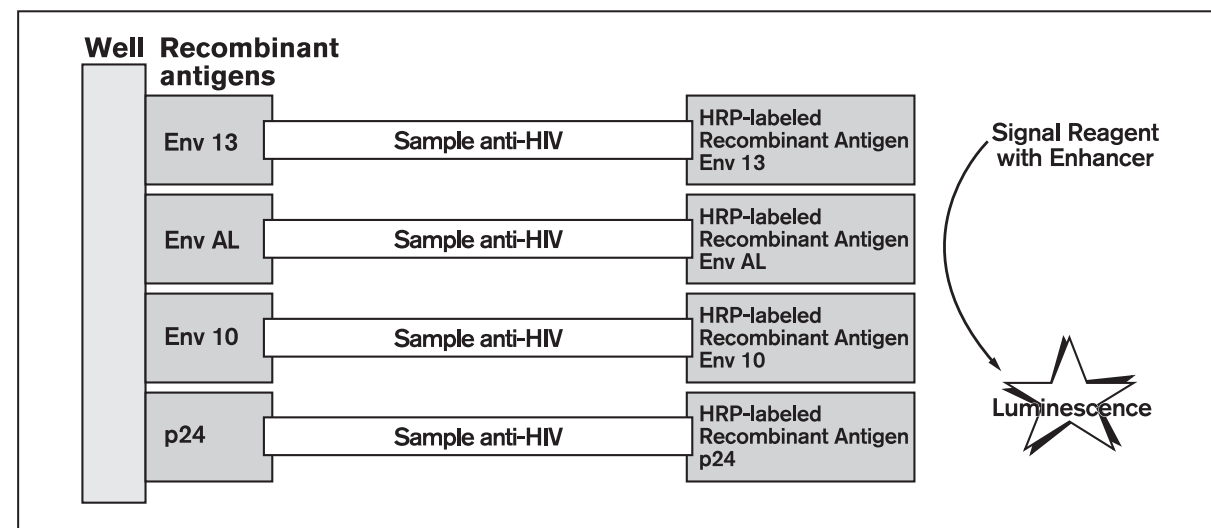
The VITROS Anti-HIV 1+2 assay is performed using the VITROS Immunodiagnostic Products Anti-HIV 1+2 Reagent Pack and the VITROS Immunodiagnostic Products Anti-HIV 1+2 Calibrator on the VITROS ECI/ECIQ Immunodiagnostic System, a random access multi-analyte immunoassay platform.

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 gp120 and gp41 regions. HIV-1 Env 10 (envelope SOD fusion protein), contains a gp41 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 gp36 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).

The assay uses an immunometric bridging technique that 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. A reagent containing luminogenic substrates (a luminal 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 luminal derivative, producing light. The electron transfer agent (a substituted acetonilide) 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+2 present.

### Reaction Scheme



ASSAY TYPE	ASSAY TIME AND TEMPERATURE	
Immunometric Assay	Incubation time:	37 minutes
	Time to first result:	48 minutes
	Temperature:	37°C

### CLINICAL STUDY POPULATIONS - OVERVIEW

The samples used in the VITROS Anti-HIV 1+2 assay clinical study were obtained from individuals at high risk for HIV infection (with or without signs or symptoms commonly associated with HIV infection), at low risk for HIV infection, and from individuals known to be HIV antibody positive. The majority of samples were collected under IRB-approved protocols from consented individuals. Specific inclusion and exclusion criteria were provided to the Principal Investigators. Demographic and/or clinical information was obtained during enrollment interviews and/or from medical records, and recorded on case report forms (CRFs). The remaining samples were obtained under IRB-approved protocols as unlinked residual samples from clinical laboratories (women during labor and delivery or in the post-partum period; pediatric subjects; insurance applicants) or from specimen archives (international HIV antibody positive).

The sensitivity and specificity of the VITROS Anti-HIV 1+2 assay were evaluated among the individuals at high risk and low risk for HIV infection. The sensitivity of the VITROS assay was further evaluated among the individuals known to be HIV antibody positive, and by testing serially collected samples from individuals with HIV infection (commercial seroconversion panels). 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.

### STUDY POPULATION DESCRIPTIONS

The study subjects were administratively divided into seven populations (and several subpopulations) to assist in sample collection, sample distribution and testing, data collection, and analysis. Table 1 provides a list of the sample populations and includes information on origin, risk or HIV status, description of included subjects, and number of subjects included in the study.

TABLE 1: VITROS ANTI-HIV 1+2 ASSAY CLINICAL STUDY SAMPLE POPULATIONS			
Population (Origin)	Risk/HIV Status	Description	Sample Size
1 (U.S.)	High	Prospective collections at multiple sites	2175 (86.7%)
2 (Ivory Coast)	High	Prospective collections at multiple sites	488 (8.2%)
3a (U.S.)	HIV Positive	Multiple sites. Clinical and laboratory documentation of HIV infection	1121 (18.9%)
3b (Int'l)	HIV Positive	Unlinked residual samples from multiple (4) geographic areas	194 (3.3%)
4 (Ivory Coast)	HIV-2 antibody positive (mono-infected)	HIV-2 antibody positive (mono-infected)	208 (3.5%)
5a (U.S.)	High	Pregnancy. Prospective collections at multiple sites	249 (4.2%)
5b (U.S.)	Low	Pregnancy. Prospective collections at multiple sites	297 (5.0%)
5c (U.S.)	Low	Pregnancy. Labor and delivery. Unlinked residual samples	49 (0.8%)
6 (U.S.)	Low	Unlinked residual samples from insurance applicants	999 (16.9%)
7a (U.S.)	Low	Pediatric subjects ages 2 - 17. Unlinked residual samples	99 (1.7%)
7b (U.S.)	HIV Positive	Pediatric subjects ages 1 - 16. Unlinked residual samples	40 (0.7%)
Total			5919 (100%)

### ASSIGNMENT OF HIV ANTIBODY STATUS TO STUDY SAMPLES

Samples from the subjects at high or low risk for HIV infection were tested with an FDA-licensed reference 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 reference and supplemental testing algorithm in Table 2. In those instances where the reference assay was negative but the VITROS assay was repeatedly reactive, supplemental testing was performed to determine the HIV antibody status of the sample.

TABLE 2: ASSIGNMENT OF HIV ANTIBODY STATUS TO STUDY SAMPLES FROM HIV HIGH AND LOW RISK SUBJECTS		
Anti-HIV 1/2 Assay Result	Supplemental Testing Result(s)	HIV Antibody Status
Negative	Not Applicable	HIV Antibody Negative
Repeatedly Reactive	Western Blot Negative	HIV Antibody Negative*
Repeatedly Reactive	Western Blot Positive	HIV Antibody Positive*
Repeatedly Reactive	Western Blot Indeterminate IFA Negative	HIV Antibody Negative*
Repeatedly Reactive	Western Blot Indeterminate IFA Positive	HIV Antibody Positive
Repeatedly Reactive	Western Blot Indeterminate IFA Indeterminate	HIV Antibody Status Not Determined*

1. 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 antibody status was "Not Determined". The HIV antibody status was "Positive" if the HIV-2 IFA was positive.  
2. 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 sensitivity of the VITROS Anti-HIV 1+2 assay was calculated as the percentage of HIV Antibody Positive subjects that tested repeatedly reactive with the VITROS assay. 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.

## Results

### COMPARISON OF VITROS AND REFERENCE ANTI-HIV 1/2 ASSAY PERFORMANCE IN HIGH RISK POPULATIONS

Samples from 2912 subjects at high risk for HIV infection were tested with the VITROS and reference anti-HIV 1/2 assays (with supplemental testing as required). The samples were obtained from subjects in the U.S. (N=2175), in the Ivory Coast (N=488) and from pregnant women in the U.S. (N=249). The results are summarized in Table 3.

TABLE 3: VITROS AND REFERENCE ANTI-HIV 1/2 ASSAY RESULTS* IN HIGH RISK POPULATIONS (N=2912)								
Population Description	Number Tested	Reference Anti-HIV 1/2 Assay			VITROS Anti-HIV 1+2 Assay			WB Positive
		NR	IR	RR	NR	IR	RR	
High Risk U.S. (Pop. 1)	2175	2106	69	68	2116	59	58	54
High Risk Ivory Coast (Pop. 2)	488	453	35	33	457	31	31	26
Pregnant High Risk U.S. (Pop. 5a)	249	244	5	4	243	6	6	5
Total	2912	2803	109	105	2816	96	95	85

1. NR = non reactive (negative); IR = initially reactive; RR = repeatedly reactive; WB = western blot

There were 85 Western Blot positive samples among the 2912 samples tested from the high risk populations (2.92%). The Western Blot positive rate for Population 1 (U.S.) was 2.48% (54/2175), for Population 2 (Ivory Coast) 5.33% (26/488), and for U.S. pregnant women at high risk for HIV infection, the rate was 2.01% (6/249).

The performance of the two assays was compared using McNemar's test method (Table 4).

TABLE 4: COMPARISON OF THE VITROS ANTI-HIV 1+2 ASSAY WITH THE REFERENCE ANTI-HIV 1/2 ASSAY IN HIGH RISK POPULATIONS (N=2912)			
VITROS Anti-HIV 1+2 Assay Result	Reference Anti-HIV 1/2 Assay Result		Total
	Repeatedly Reactive	Negative	
Repeatedly Reactive	84	11	95
Negative	21	2796	2817
Total	105	2807	2912
P-Value (McNemar's Test)			0.0771

The p value for McNemar's test was 0.0771, which indicated there was no statistically significant difference in performance between the two assays.

Tables 5 and 6 present the agreement of the VITROS and reference assays, respectively, with HIV antibody status in high risk populations.

TABLE 5: AGREEMENT OF THE VITROS ANTI-HIV 1+2 ASSAY WITH HIV ANTIBODY STATUS IN HIGH RISK POPULATIONS (N=2912)				
VITROS Anti-HIV 1+2 Assay Result	HIV Antibody Status			Total
	Positive	Negative	Not Determined <sup>1</sup>	
Repeatedly Reactive	85	5	5	95
Negative	0	2812	5	2817
Total	85	2817	10	2912

1. These samples remained "HIV status Not Determined" following VITROS, reference and supplemental assay testing for anti-HIV-1 and anti-HIV-2 (EIA, WB, IFA). They were considered anti-HIV negative when calculating sensitivity and specificity.

TABLE 6: AGREEMENT OF THE REFERENCE ANTI-HIV 1/2 ASSAY WITH HIV ANTIBODY STATUS IN HIGH RISK POPULATIONS (N=2912)				
Reference Anti-HIV 1/2 Assay Result	HIV Antibody Status			Total
	Positive	Negative	Not Determined <sup>1</sup>	
Repeatedly Reactive	83	17	5	105
Negative	2	2800	5	2807
Total	85	2817	10	2912

1. These samples remained "HIV status Not Determined" following VITROS, reference and supplemental assay testing for anti-HIV-1 and anti-HIV-2 (EIA, WB, IFA). They were considered anti-HIV negative when calculating sensitivity and specificity.

The sensitivity of the VITROS Anti-HIV 1+2 assay in high risk populations was 100% (85/85) with a 95% exact confidence interval (CI) of 95.75% to 100.0% compared to 97.65% (83/85) for the reference anti-HIV 1/2 assay.

The specificity of the VITROS Anti-HIV 1+2 assay in high risk populations was 99.65% (2817/2827; CI = 99.35% to 99.83%) compared to 99.22% (2805/2827) for the reference anti-HIV 1/2 assay.

### COMPARISON OF VITROS AND REFERENCE ANTI-HIV 1/2 ASSAY PERFORMANCE IN LOW RISK POPULATIONS

Samples from 1444 subjects at low risk for HIV infection were tested with the VITROS and reference anti-HIV 1/2 assays (with supplemental testing as required). The 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). The results are summarized in Table 7.

TABLE 7: VITROS AND REFERENCE ANTI-HIV 1/2 ASSAY RESULTS* IN LOW RISK POPULATIONS (N=1444)							
Population Description	Number Tested	Reference Anti-HIV 1/2 Assay			VITROS Anti-HIV 1+2 Assay		WB Positive
		NR	IR	RR	NR	IR	
Pregnant Low Risk U.S. (Pop. 5b)	297	295	2	2	294	3	1
Labor & Delivery Low Risk U.S. (Pop. 5c)	49	49	0	0	48	1	0
Insurance Applicants Low Risk U.S. (Pop. 6)	999	993	6	6	991	8	7
Pediatric Low Risk U.S. (Pop. 7a)	99	99	0	0	99	0	0
Total	1444	1436	8	8	1432	12	9

1. NR = non reactive (negative); IR = initially reactive; RR = repeatedly reactive; WB = western blot

There were six Western Blot positive samples among the 1444 low risk samples tested (0.42%). One was found among the 297 U.S. pregnant women at low risk for HIV infection (Population 5b; 0.34%), and five among the 999 insurance applicants (Population 6; 0.50%). There were no Western Blot positive results in women in the period around labor and delivery or in the pediatric subjects (Populations 5c and 7a, respectively).

The performance of the two assays was compared using McNemar's test method (Table 8).

TABLE 8: COMPARISON OF THE VITROS ANTI-HIV 1+2 ASSAY WITH THE REFERENCE ANTI-HIV 1/2 ASSAY IN LOW RISK POPULATIONS (N=1444)			
VITROS Anti-HIV 1+2 Assay Result	Reference Anti-HIV 1/2 Assay Result		Total
	Repeatedly Reactive	Negative	
Repeatedly Reactive	2	7	9
Negative	6	1429	1435
Total	8	1436	1444
P-Value (McNemar's Test)			0.7815

The p value of McNemar's test was 0.7815, which indicated there was no statistically significant difference in performance between the two assays.

Tables 9 and 10 present the agreement of the VITROS and reference assays, respectively, with HIV antibody status in low risk populations.

TABLE 9: 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 Result	HIV Antibody Status			Total
	Positive	Negative	Not Determined <sup>1</sup>	
Repeatedly Reactive	6	2	1	9
Negative	0	1435	0	1435
Total	6	1437	1	1444

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

TABLE 10: AGREEMENT OF THE REFERENCE ANTI-HIV 1/2 ASSAY WITH HIV ANTIBODY STATUS IN LOW RISK POPULATIONS (N=1444)				
Reference Anti-HIV 1/2 Assay Result	HIV Antibody Status			Total
	Positive	Negative	Not Determined <sup>1</sup>	
Repeatedly Reactive	2	6	0	8
Negative	4	1431	1	1436
Total	6	1437	1	1444

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

The sensitivity of the VITROS Anti-HIV 1+2 assay in low risk populations was 100% (6/6; CI = 54.07% to 100.0%) compared to 33.33% (2/6) for the reference anti-HIV 1/2 assay.

The specificity of the VITROS Anti-HIV 1+2 assay in low risk populations was 99.79% (1435/1438; CI = 99.39% to 99.96%) compared to 99.58% (1432/1438) for the reference anti-HIV 1/2 assay.

### SENSITIVITY IN KNOWN HIV ANTIBODY POSITIVE POPULATIONS

A total of 1563 samples from known HIV antibody positive subjects was tested with the VITROS Anti-HIV 1+2 assay. These samples were obtained from subjects in the U.S. (N=1121), from four geographic locations outside the U.S. (N=194), from mono-infected HIV-2 antibody positive subjects in the Ivory Coast (N=208) and from HIV antibody positive pediatric subjects, ages 1-16 years, in the U.S. (N=40). The results are summarized in Table 11.

Description (Population)	N	VITROS Anti-HIV 1+2 Assay		
		NR	IR	RR
HIV Positive - U.S. (Pop. 3a)	1121	0	1121	1121
HIV Positive - Int'l (Pop. 3b)	194	1 <sup>1</sup>	193	193
HIV-2 Positive - Ivory Coast (Pop. 4)	208	0	208	208
HIV Positive Pediatric (Pop. 7b)	40	0	40	40
Total	1563	1	1562	1562

1. This archived sample was obtained from a rural clinic in Kaffi, 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 reference 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.

The VITROS Anti-HIV 1+2 assay was repeatedly reactive in 99.94% (1562/1563) of the HIV antibody positive samples. One archived sample obtained from Africa was not detected by the VITROS assay.

CD4+ counts were available for 1094 of the 1121 HIV antibody positive subjects from the U.S. (Population 3a). The VITROS Anti-HIV 1+2 assay was repeatedly reactive with all 1121 samples regardless of the subjects' CD4+ counts (Table 12).

TABLE 12: PERFORMANCE OF THE VITROS ANTI-HIV 1+2 ASSAY AMONG HIV INFECTED U.S. SUBJECTS WITH DOCUMENTED CD4+ COUNTS			
CD4+ Count	N	VITROS Anti-HIV 1+2 Assay Result	
		IR	RR
<200	149	149	149
200 - 499	429	429	429
≥499	516	516	516
Unknown	27	27	27
Total	1121	1121	1121

### SENSITIVITY AND SPECIFICITY OF THE VITROS ANTI-HIV 1+2 ASSAY BY STUDY POPULATION

The sensitivity and/or specificity of the VITROS Anti-HIV 1+2 assay in the high risk, low risk and known HIV antibody positive populations tested in this clinical study are presented in Table 13.

TABLE 13: SENSITIVITY AND SPECIFICITY OF THE VITROS ANTI-HIV 1+2 ASSAY BY STUDY POPULATION				
Population	Sensitivity	95% Exact Confidence Intervals	Specificity	95% Exact Confidence Intervals
High Risk Ivory Coast (Pop. 2)	100% (26/26)	86.77% - 100%	98.92% (457/462)	97.49% - 99.65%
HIV Positive - U.S. (Pop. 3a)	100% (1121/1121)	99.67% - 100%		
HIV Positive - Int'l (Pop. 3b)	99.48% (193/194)	97.16% - 99.99%		
HIV-2 Positive Ivory Coast (Pop. 4)	100% (208/208)	98.24% - 100%		
Pregnant High Risk U.S. (Pop. 5a)	100% (6/5)	47.82% - 100%	99.59% (243/244)	97.74% - 99.99%
Pregnant Low Risk U.S. (Pop. 5b)	100% (1/1)	2.50% - 100%	100% (296/296)	98.76% - 100%
Labor & Delivery Low Risk U.S. (Pop. 5c)	100% (48/49)		97.96% (48/49)	89.15% - 99.95%
Insurance Applicants Low Risk U.S. (Pop. 6)	100% (5/5)	47.82% - 100%	99.80% (992/994)	99.28% - 99.98%
Pediatric Low Risk U.S. (Pop. 7a)	100% (99/99)		100% (99/99)	96.34% - 100%
HIV Positive Pediatric (Pop. 7b)	100% (40/40)	91.19% - 100%		
Total	99.94% (1653/1654)	99.66% - 100%	99.70% (4252/4265)	99.48% - 99.84%

With the exception of the HIV Antibody Positive archived samples from various locations outside the U.S. (Population 3b), sensitivity of the VITROS Anti-HIV 1+2 assay was 100.0%. The sensitivity for Population 3b was 99.48% (193