SUMMARY OF SAFETY AND EFFECTIVENESS

Product: VITROS Immunodiagnostic Products HIV Combo Reagent Pack and VITROS Immunodiagnostic Products HIV Combo Calibrator (VITROS HIV Combo test)

I. General Information

Device Generic Name: HIV-1/2 Ag/Ab Test

Device Trade Name: VITROS Immunodiagnostic Products HIV Combo Reagent Pack and VITROS Immunodiagnostic Products HIV Combo Calibrator (VITROS HIV Combo test)

Applicant's Name and Address: Ortho-Clinical Diagnostics, Inc.
100 Indigo Creek Drive
Rochester, NY 14626

Manufacturer: Ortho-Clinical Diagnostics
Felindre Meadows, Pencoed
Bridgend CF35 5PZ
United Kingdom
Registration Number: 3007111389

Premarket Approval Application (PMA) Number: BP 160122

Date of Panel Recommendation: Not Applicable

Office's Signatory Authority: Jay S. Epstein, M.D.

Director, OBRR/CBER

□ I concur with the summary review.

□ I concur with the summary review and include a separate review to add further analysis.

Date of Notice of Approval to the Applicant:

Material Reviewed/Consulted: The PMA, amendments to the PMA, and other specific documentation used in developing the Summary of Safety and Effectiveness (SSE)

Review memos from the following reviewers were used in developing the SSE:

Reviewer Name	**Discipline Reviewed/Responsibility**
Pawan K. Jain	Scientific Lead Clinical Labeling
Julia Lathrop	Clinical
Krishnakumar Devadas	Pre-Clinical, CMC (Chemistry, Manufacturing, and Controls)
Xue Wang	Preclinical
Bhanu Kannan	Bioresearch Monitoring
Cecily Jones	Facility and GMP
Sajjad Syed	Instrument and Software
Zhen Jiang	Statistical
Dana Jones	Labeling

II. Intended Use

VITROS Immunodiagnostic Products HIV Combo Reagent Pack is for the simultaneous qualitative detection of antibodies to Human Immunodeficiency Virus types 1, including group M and O, and/or 2 (anti-HIV-1 and anti-HIV-2) and HIV p24 antigen in human serum and plasma (heparin and EDTA) in adults, pregnant women, adolescents and children (as young as 2 years of age), using the VITROS 3600 Immunodiagnostic System.

A reactive result with the VITROS HIV Combo test does not distinguish between the detection of HIV-1 p24 antigen, antibodies to HIV-1, and antibodies to HIV-2.

The results of the VITROS HIV Combo 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. The VITROS HIV Combo assay is not intended for use in screening blood or plasma donors. The effectiveness of the VITROS HIV Combo assay for use in screening blood, plasma, cell or tissue 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.

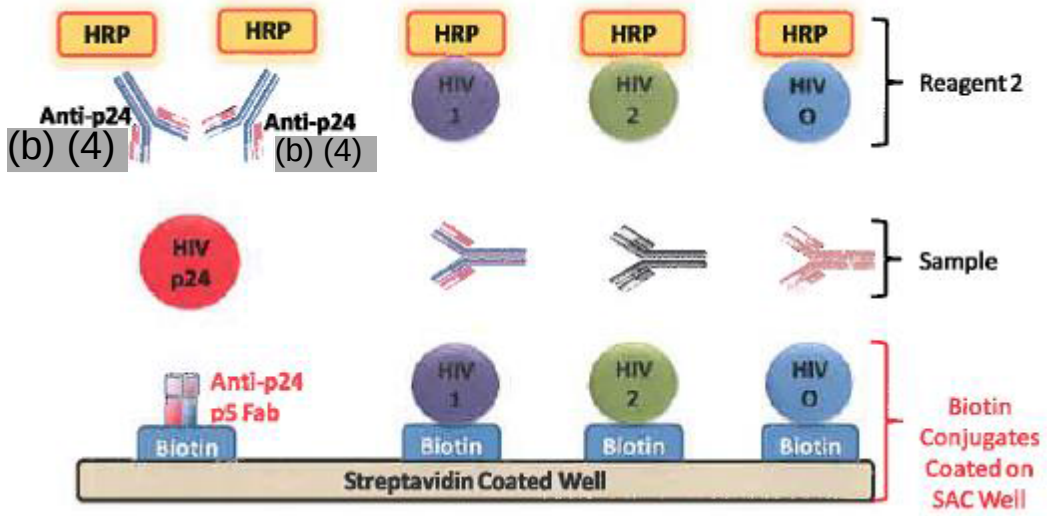
It is not intended for newborn screening or for use with cord blood specimens or specimens from individuals less than 2 years of age.

VITROS Immunodiagnostic Products HIV Combo Calibrator is for use in the calibration of the VITROS 3600 Immunodiagnostic System with the VITROS HIV Combo Reagent Pack.

III. Device Description

The VITROS HIV Combo test uses 3 recombinant antigens derived from HIV-1 envelope (env 13; containing (b) (4) sequence of (b) (4), HIV-1 group O envelope (env 70-3; derived from HIV-1 Group O (b) (4) strain (b) (4) and HIV-2 envelope (env 31; contains a (b) (4) transmembrane region). These antigens detect antibodies to HIV-1 and HIV-2 in the same test. The use of these recombinant antigens improves test specificity by avoiding nonspecific reactions due to cross-reaction with human cell proteins which are present in cell lysates. The VITROS HIV Combo test also uses antibodies to HIV-1 p24 antigen to enable detection of HIV-1 p24 antigen that may be present earlier than the onset of the antibody response enabling earlier diagnosis of HIV-1 infection. For the detection of HIV-1 p24 antigen in test samples, biotinylated fragments of mouse monoclonal antibody capture HIV-1p24 antigen, which reacts with two HRP conjugated mouse monoclonal antibodies to produce the signal.

The following is a schematic representation of the complexes formed in the device:

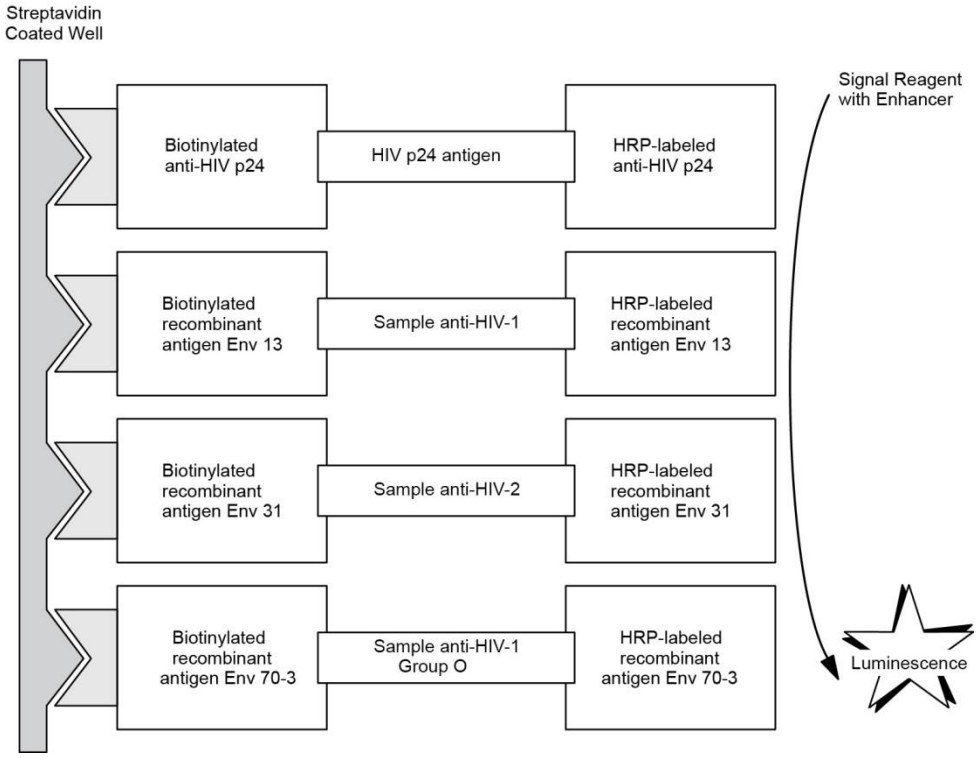


The VITROS HIV Combo test is performed using the VITROS HIV Combo Reagent Pack and the VITROS HIV Combo Calibrator on the VITROS 3600 Immunodiagnostic System that uses Intellicheck® Technology. An immunometric technique is used for the assay; this involves a two-stage reaction. In the first stage, HIV antibody or antigen present in the sample bind to biotinylated HIV recombinant antigen or biotinylated antibody immobilized on streptavidin coated wells. Unbound sample is removed by washing. In the second stage, conjugate reagent containing horseradish peroxidase (HRP)-labeled recombinant HIV antigens and antibodies is added. The conjugate binds specifically to any human anti-HIV-1 or anti-HIV-2 (IgG and IgM) or HIV antigen 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 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 system.

The sample volume used is 80 µl, incubation time is 37 minutes, and time to first result is 48 minutes.

The following is the schematic presentation of the reaction to produce a light signal:



Components of the VITROS HIV Combo test

Materials Provided

- VITROS Immunodiagnostic Products HIV Combo Reagent Pack (VITROS HIV c Reagent Pack) and VITROS Immunodiagnostic Products HIV Combo Calibrator (VITROS HIV c Calibrator) together comprise the VITROS HIV c test.

- VITROS HIV c Reagent Pack Contents

    - 100 coated wells (streptavidin, bacterial; binds ≥ 3 ng biotin/well) (biotin-mouse monoclonal anti-HIV p24, 0.3 µg/mL and biotin-recombinant HIV antigens, 0.1025 µg/mL)
    - 6.2 mL assay reagent (buffer with bovine gamma globulin, bovine serum albumin and antimicrobial agent)
    - 16.2 mL conjugate reagent (HRP-recombinant HIV antigens, 0.021-0.266 µg/mL and HRP-mouse monoclonal anti-HIV p24, 1.5 µg/mL) in buffer with goat serum, bovine serum albumin and antimicrobial agent

- VITROS HIV c Calibrator Contents:

    - 2.0 mL VITROS HIV c Calibrator (anti-HIV-1 positive human plasma in anti-HIV 1+2 negative human plasma) with antimicrobial agent

- Additional Required Components (sold separately):

- Controls containing anti-HIV-1, anti-HIV-2, anti-HIV-1 group O, and HIV p24 antigen are recommended for monitoring the VITROS HIV Combo test, and used according to the instructions provided.
- VITROS 3600 Immunodiagnostic Systems (VITROS 3600).
- VITROS Immunodiagnostic Products Signal Reagent and VITROS Immunodiagnostic Products Universal Wash Reagent.

IV. Quality Control of Manufacturing

VITROS HIV Combo test components such as VITROS Immunodiagnostic Products HIV Combo Reagent Pack (VITROS HIV c Reagent Pack) and VITROS Immunodiagnostic Products HIV Combo Calibrator (VITROS HIV c Calibrator) are manufactured at Ortho-Clinical Diagnostics, Felindre Meadows, Pencoed, Bridgend, South Wales, United Kingdom, CF35 5PZ.

Reagents used in the manufacture of the assay such as biotinylated recombinant antigen or antibody coated on the streptavidin wells, and horseradish peroxidase (HRP)-labeled recombinant antigens and antibodies are manufactured by Ortho-Clinical Diagnostics, Inc. or obtained from vendors certified by Ortho-Clinical Diagnostics. Acceptance criteria and testing specifications have been pre-established for all kit components and reagents by Ortho-Clinical Diagnostics and approved by FDA. Every lot of the kit components and reagents must meet testing specifications to be acceptable for use in further manufacturing. Each lot of Reagent Pack and Calibrator is subjected to a final performance test with a panel of samples containing (b) (4) of HIV-1 p24 antigen, anti-HIV-1 antibodies, and anti-HIV-2 antibodies. Every lot of VITROS HIV Combo test must meet the established performance specifications prior to release by Ortho Clinical Diagnostics.

V. Stability

To assess the stability of the VITROS HIV Combo test up to the (b) (4) week time-point, data from runs performed using Reagent Packs and Calibrators stored at 2-8°C from (b) (4) Lots were used. (b) (4) Lots (b) (4) were tested at weeks 0, 4, 8, (b) (4).

The Reagent Packs and Calibrators were subjected to simulated transport conditions before starting the stability trial. To simulate transport conditions, Reagent Packs and Calibrators were stored at (b) (4) for (b) (4) and then returned to 2-8°C prior to testing.

(b) (4) runs were performed at each time point for each Kit Lot. Each run contained duplicate determinations of calibrators, and a single determination of controls.

The data show the stability of the VITROS HIV Combo Reagent Pack and Calibrator is within trending limits and acceptable up to the (b) (4) week time point. The data, when used in conjunction with the Preservative Efficacy test results, support a stability claim of up to (b) (4) weeks.

VI. Random Access

The VITROS HIV Combo test can be performed either in random access or batch mode. Studies were performed to show that precision and accuracy performance are consistent between the two modes. This included running (b) (4) tests interspersed with VITROS HIV Combo in random access mode and comparing results to batch mode processing. The test samples selected for testing included a (b) (4) for HIV p24 antigen and HIV-1 group M, HIV-2, HIV-1 group O antibodies). In addition, (b) (4) was included to challenge the system with a worst case for sample carryover.

The data demonstrate that testing VITROS Immunodiagnostic Products HIV Combo Reagent Pack and VITROS Immunodiagnostic Products HIV Combo Calibrators on VITROS 3600 Immunodiagnostic Systems in random access mode causes no shift in the results of samples compared with running in batch mode.

VII. Testing Procedures and Interpretation of Results

a. Specimens collection and preparation

Serum samples and plasma samples collected in lithium heparin, sodium heparin and potassium EDTA tubes are recommended for testing using the VITROS HIV Combo test, and are collected using standard procedures.

Samples are kept in stoppered containers to avoid contamination and evaporation. Samples are brought to 15-30°C (59-86°F) before use and should be returned to 2-8°C as soon as possible after use. Samples may be stored up to 24 hours at room temperature (up to 30°C, 86°F) and up to 7 days at 2-8°C (36-46°F).

NOTE: Samples that will not be tested within the recommended time frames as outlined above should be stored at -20°C (-4°F) for up to six months and may be subjected to up to five freeze-thaw cycles.

b. Calibration

The VITROS HIV Combo Calibrator is supplied ready for use. The Calibrator and Reagent Pack lot numbers are linked for use. The Calibrator can only be used with a linked lot number of the Reagent Pack.

Calibration is required after a pre-determined interval, or when a different reagent lot is loaded. Calibration results are assessed against a range of quality parameters. Failure to meet any of the defined quality parameter ranges are coded in the calibration report. Following a failed calibration, a required action is taken as described in the system's operator's manual. In addition, calibration is performed every 28 days, after a specified service procedure, and if quality control results are consistently outside the acceptable range.

c. Quality Control

Controls containing suitable levels of anti-HIV-1, anti-HIV-2, anti-HIV-1 group O, and HIV p24 antigen are recommended for use with the VITROS 3600 Immunodiagnostic System.

The following controls have been tested and found suitable for use:

Vendor	Product	Control Level
Bio-Rad	VIROCLEAR®	negative control
	VIROTROL®I	anti-HIV-1 positive
	VIROTROL® HIV-2	anti-HIV-2 positive
	VIROTROL® HIV-1 gO	anti-HIV-1 gO positive
	VIROTROL® HIV-1 Ag	p24 antigen positive

The performance of other commercial or non-commercial controls should be evaluated for compatibility with this test before they are used for quality control.

Control materials may show a difference when compared with other HIV Ag/Ab assays if they contain high concentrations of preservatives, stabilizers, other non-physiological additives, or otherwise depart from a true human sample matrix. Appropriate quality control value ranges must be established for all quality control materials used with the VITROS HIV Combo test.

To verify system performance, control materials are analyzed after calibration, according to local requirements such as laboratory SOPs and other testing specifications, or at least once each day while the test is being performed, and after specified service procedures.

d. Interpretation of Results

The final interpretation of results obtained with the VITROS HIV Combo test on the VITROS 3600 Immunodiagnostic System is summarized in the table below:

Initial VITROS HIV Combo test Results (S/C)	Action	Duplicate Retest Results (S/C)	Final Interpretation
< 1.00	None	Not Applicable	Negative Sample is negative for anti-HIV-1 (including Group O), anti-HIV-2 and p24 antigen
≥ 1.00	Retest in duplicate	Both < 1.00	Negative Sample is negative for anti-HIV-1 (including Group O), anti-HIV-2 and p24 antigen

≥ 1.00	Retest in duplicate	One or both ≥ 1.00	Reactive Sample is reactive for anti-HIV-1 (including Group O), and/or anti-HIV-2 and/or p24 antigen

If the final interpretation for a sample is reactive, the probability that HIV antibodies or antigen are present is high and it is appropriate to investigate by additional, more specific tests. Samples found reactive by the VITROS HIV Combo test and positive by additional, more specific tests are considered positive for antibodies to HIV-1 (including Group O), and/or HIV-2, and/or p24 antigen. Clinical correlation is indicated with appropriate counseling, medical intervention, and possibly additional testing to decide whether a diagnosis of HIV infection is accurate.

If interpretation of results from samples found to be reactive by the VITROS HIV Combo test and negative by additional, more specific tests is unclear, further clarification may be obtained by collecting and testing additional follow-up samples.

The antigen antibody reaction has its own complexity such as avidity and affinity of the antibodies, therefore, the magnitude of a VITROS HIV Combo test signal (e.g., OD or S/C) cannot be correlated to an endpoint titer.

VIII. Limitations and Restriction of the Test

- The Vitros HIV-1/2 Ag/Ab Test is for in vitro diagnostic use only.
- This test is not approved for the routine screening of blood, plasma, cell or tissue donors.
- This assay has not been evaluated for newborn screening or for use with cord blood specimens or for use with specimens from individuals less than 2 years of age.
- The results from this or any other diagnostic test should be used and interpreted only in the context of the overall clinical picture.
- Heterophilic antibodies in serum or plasma samples may cause interference in immunoassays. 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.
- Any testing material including quality control material preserved in azide should not be used.
- This assay may not give reactive test results in all infected individuals, therefore, a negative test results does not exclude the possibility of exposure or infection with HIV. HIV antibodies and/or p24 antigen may be undetectable in some stages of the infection and in some clinical conditions.
- An individual who has antigen or antibodies to HIV is presumed to be infected with the virus. However, an individual who has received an investigational HIV vaccine may develop antibodies to the vaccine and may or may not be infected with HIV.

IX. Warnings

1. Read the Package Insert completely before using this assay. Follow the instructions carefully as not doing so may result in inaccurate test results.

2. Any product content that is visibly damaged should not be used.

3. Use caution when handling material of human origin. Consider all samples as potentially infectious. No test method can offer complete assurance that hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV 1+2) or other infectious agents are absent. Handle, use, store and dispose of solid and liquid waste from samples and test components, in accordance with procedures defined by appropriate national biohazard safety guideline or regulation (e.g., CLSI document M29).

4. The HIV antibody positive plasma has been treated 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

5. The VITROS HIV Combo Reagent Pack contains 1.0% ProClin 300, and can cause an allergic skin reaction. Wear protective gloves/protective clothing/eye protection/face protection. If contact with skin, wash with plenty of soap and water. If skin irritation or rash occurs, get medical advice/attention. Wash contaminated clothing before reuse.

6. The VITROS HIV Combo Calibrator contains 1.0% ProClin 950, that can cause skin irritation or an allergic skin reaction or serious eye irritation. Wear protective gloves/protective clothing/eye protection/face protection. If splashed into Eyes: Rinse cautiously with water for several minutes. If contact lenses are present, remove them if easy to do so. Continue rinsing. If eye irritation persists, get medical advice/attention for specific treatment.

7. Use of this test with specimen types other than those specifically approved for use with this device may produce inaccurate test results.

8. This test should be performed at 15 to 30°C (59° to 86°F). If stored refrigerated, ensure that the specimens are brought to the operating temperature before performing testing.

9. Do not use test contents beyond labeled expiration date.

X. Alternative Practices and Procedures

In a test, sample detection of antibodies against HIV epitopes and HIV-1 p24 antigen can be done in a variety of ways, including enzyme or chemiluminescent immunoassays. Tests may be intended for laboratory use by laboratory professionals, at the point-of-care by healthcare professionals, or for home use by lay persons.

XI. Marketing History

A previous version of this device (part # (b) (4) has been marketed exclusively outside the United States and is currently available in Europe and countries accepting the CE Mark.

XII. Potential Adverse Effects of the Device on Health

Potential adverse effects of the VITROS HIV Combo test relate to the risk of false positive and false negative results. While performance studies indicate that this risk is likely to be very small, the potential for inaccurate results exists. The risk of incorrect results is minimized by following the procedures and instructions outlined in the Package Insert.

XIII. Summary of Preclinical Studies

a. HIV-1 p24 Analytical Sensitivity

HIV-1 p24 antigen analytical sensitivity was determined by testing serial dilutions of the WHO HIV-1 p24 Antigen Dilution standard (NIBSC code 90/636) and the AFSSAPS HIV-1 p24 Antigen Dilution standard using two reagent lots and three instruments. One reagent lot was used with two instruments and the other lot was used with one instrument. The values for three determinants were 0.46, 0.45, and 48 IU/mL for the WHO standard, and 12.6, 12.7 and 13.1 pg/mL for the AFSSAPS standard. These results demonstrated a mean sensitivity for HIV-1 p24 antigen of 0.46 IU/mL (range 0.45 to 0.48 IU/mL) for the WHO HIV-1 p24 Antigen standard and 12.8 pg/mL (range 12.6 to 13.1 pg/mL) for the AFSSAPS HIV-1 Antigen standard.

b. Detection of HIV Antigen Genotypes

Three HIV-1 group M subtype specimens and 49 viral isolates were tested with the VITROS HIV Combo test. The HIV-1 antigen subtype and country of origin for each isolate, as well as the reactivity for these samples on the VITROS 3600 Immunodiagnostic System and an FDA approved HIV Ag/Ab combo test, are presented in Table 1.

Table 1: Detection of HIV Antigen Genotypes

			VITROS HIV Combo test	**Approved Ag/Ab Combo Test**
Source (# of Specimens)	**Subtype**	**Number of Specimens Tested**	**Number of Reactive Interpretations**	**Number of Reactive Interpretations**
Group M Specimens (3)				
Uganda	A	1	1	1
Uganda	D	1	1	1
Romania	F	1	1	1
Viral Isolates* (49)				
Ghana (1), Kyrgyzstan (1), Uganda (2)	A	4	4	4
Brazil (1), Thailand (1), USA (6)	B	8	8	7
Djibouti (1), Ethiopia (1), Senegal (1), Somalia (1), Uganda (1), Zambia (1), Unknown (1)	C	7	5	5

			VITROS HIV Combo test	Approved Ag/Ab Combo Test
Source (# of Specimens)	**Subtype**	**Number of Specimens Tested**	**Number of Reactive Interpretations**	**Number of Reactive Interpretations**
Senegal (1), Uganda (2)	D	3	3	3
Brazil (3), Romania (2)	F	5	5	5
Kenya (1), Democratic Republic of the Congo (1)	G	2	2	1
Democratic Republic of the Congo (1)	H	1	1	1
Indonesia (2), Thailand (8)	CRF01_AE	10	10	10
Djibouti (2), Liberia (1)	CRF02_AG	3	3	3
Cameroon (2), USA (1), Spain (1)	Group O	4	4	4
Unknown	IIIB	1	1	0
Unknown	HIV-2	1	1	1
Totals		**52**	**50 (96.2)**	**47**

*Isolates tested after dilution to 200,000 RNA copies/mL in defibrinated, delipidized plasma.

Test results obtained using the VITROS HIV Combo test are comparable to the FDA approved Ag/Ab combo test, however, in this study the VITROS HIV Combo test detected additional subtype B, G and IIIB specimens compared to the approved HIV Ag/Ab Combo test.

c. Seroconversion Panels

Thirty-two commercially available seroconversion panels were tested on both the VITROS HIV Combo test and an approved HIV Ag/Ab combo test. Results for the thirty-two panels are summarized in Table 2, which presents the days elapsed from the date of the initial bleed to the last non-reactive sample and first reactive sample and the difference in days to the first reactive result between the two tests. The test results for both the assays are in agreement for 25 of the 32 panels. The approved assay was reactive two bleeds earlier (7 days) for a single panel (PRB965). For the remaining 6 of the 7 panels with discrepant results, the VITROS HIV Combo test was reactive one bleed earlier (2-7 days) than the approved Ag/Ab Combo test.

Table 2: Days to Evidence of HIV Infection

Panel ID	Number of Panel Members Tested	Number of Reactive Panel Members		Approved HIV Ag/Ab Combo Test		VITROS HIV Combo test		Difference in Days to First Reactive Result
		Approved HIV Ag/Ab Combo Test	VITROS HIV Combo test	Day of Last Non-reactive Result[1]	Day of First Reactive Result[2]	Day of Last Negative Result[1]	Day of First Reactive Result[2]	Licensed Test minus VITROS HIV Combo test
PRB926	6	4	4	2	7	2	7	0
PRB939(E)	9	4	4	14	16	14	16	0
PRB942	4	1	1	9	14	9	14	0
PRB943	**7**	5	5	5	7	5	7	0
PRB944	6	4	4	2	7	2	7	0
PRB945	6	3	4	7	13	3	7	6
PRB946	4	2	2	4	7	4	7	0
PRB947	4	3	3	0	9	0	9	0
PRB948	4	1	1	20	23	20	23	0
PRB949	4	1	1	9	18	9	18	0
PRB950	4	2	3	18	21	0	18	3
PRB951	6	4	4	2	8	2	8	0
PRB953	4	2	3	3	7	0	3	4
PRB954	7	2	2	14	17	14	17	0
PRB955	5	4	4	0	3	0	3	0
PRB956	5	2	2	42	47	42	47	0
PRB958	6	4	4	2	7	2	7	0
PRB960	9	2	2	21	28	21	28	0
PRB961	9	2	2	21	27	21	27	0
PRB962	6	2	2	9	14	9	14	0
PRB963	7	2	2	14	17	14	17	0
PRB965	6	5	3	0	5	7	12	-7
PRB969	10	3	4	63	70	61	63	7
HIV6247	10	4	4	16	21	16	21	0
HIV6248	7	2	2	14	18	14	18	0
HIV9013	7	1	2	23	25	18	23	2
HIV9015	8	2	2	21	30	21	30	0
HIV9016	10	2	2	27	30	27	30	0
HIV9021	17	4	4	43	47	43	47	0
HIV9028	7	2	2	34	53	34	53	0
HIV9032	14	7	8	22	24	17	22	2
HIV12008	13	5	5	23	28	23	28	0

Panel ID	Number of Panel Members Tested	Number of Reactive Panel Members		Approved HIV Ag/Ab Combo Test		VITROS HIV Combo test		Difference in Days to First Reactive Result
		Approved HIV Ag/Ab Combo Test	VITROS HIV Combo test	Day of Last Non-reactive Result[1]	Day of First Reactive Result[2]	Day of Last Negative Result[1]	Day of First Reactive Result[2]	Licensed Test minus VITROS HIV Combo test
Total	231	93	97	504	668	474	651	17

[1] post bleed day of last non-reactive (negative), results, usually denotes previous bleed from first reactive result result, usually denotes previous bleed from first reactive result.

[2] post bleed day of first reactive result.

Test results obtained by using the VITROS HIV Combo test are comparable to the FDA approved Ag/Ab combo test.

d. Reactivity with HIV-1 (b) (4)

(b) (4) were tested in (b) (4) using (b) (4) VITROS HIV Combo reagent lots. Of the (b) (4) tested there were (b) (4) in which the VITROS (b) (4) was greater than a licensed HIV Ag/Ab combo test, (b) (4) in which the (b) (4) of a licensed HIV Ag/Ab combo test was greater than the VITROS, and (b) (4) in which the (b) (4) of the two tests was equivalent. The overall results indicate equivalence of (b) (4) between the VITROS HIV Combo test and the FDA approved HIV Ag/Ab combo test.

Test results obtained by using the VITROS HIV Combo test are comparable to the FDA approved Ag/Ab combo test.

e. Interference by Unrelated Medical Conditions

The VITROS HIV Combo test was evaluated for potential cross-reactivity with HIV negative samples from medical conditions other than infection with HIV. The results are summarized in Table 3.

Table 3: Summary of VITROS HIV Combo test results with Potentially Cross-Reacting Samples

Sample Category	Number Tested	Number Negative	Number Reactive	Number Confirmed Positive#
HCV Antigen	6	6	0	NA
HCV Antibody	10	10	0	NA
HBsAg	6	6	0	NA

Sample Category	Number Tested	Number Negative	Number Reactive	Number Confirmed Positive#
HBc Antibody	10	10	0	NA
HTLV I Antigen	6	6	0	NA
HTLV II Antigen	6	6	0	NA
HTLV I Antibody	6	6	0	NA
HTLV II Antibody	6	6	0	NA
EBV Antigen	6	6	0	NA
EBV Antibody	10	10	0	NA
HSV I Antigen	6	6	0	NA
HSV II Antigen	6	6	0	NA
HSV Antibody	12	12	0	NA
Chlamydia	10	10	0	NA
Gonorrhea	10	10	0	NA
Syphilis	10	10	0	NA
Multiparous Female	10	10	0	NA
Pregnancy (1st Trimester)	8	8	0	NA
Pregnancy (2nd Trimester)	8	8	0	NA
Pregnancy (3rd Trimester)	8	8	0	NA
Pre Flu Vaccine	6	5	1	1
Post Flu Vaccine	6	5	1	1
Influenza A Antigen	6	6	0	NA
Influenza B Antigen	6	6	0	NA
Rheumatoid Factor (RF)	11	11	0	NA
Human Anti-Mouse Antibody (HAMA)	10	10	0	NA
Autoimmune Disease	10	10	0	NA
Anti-Nuclear Antibodies (ANA)	10	10	0	NA
Hemophilia	20	11	9	9
Dialysis	10	10	0	NA
Yeast Reactive: Candida	10	10	0	NA
Super-oxide Dismutase (SOD)	1	1	0	NA
Cytomegalovirus Antigen	6	6	0	NA
Cytomegalovirus Antibody	10	10	0	NA
Toxoplasma infection	10	10	0	NA
HAV Antigen	6	6	0	NA
HAV Antibody	10	10	0	NA
Rubella infection	10	10	0	NA
Elevated IgG	22	21	1	1
Elevated IgM	10	10	0	NA
Non-Viral Liver Disease	10	10	0	NA
Pediatric (2-5 yrs.)	4	4	0	NA
Pediatric (6-10 yrs.)	6	6	0	NA

Sample Category	Number Tested	Number Negative	Number Reactive	Number Confirmed Positive#
Pediatric (11-16 yrs.)	6	6	0	NA
Pediatric (17-21 yrs.)	4	4	0	NA
Elevated Cholesterol	21	20	1	1
Elevated Total Protein	14	10	4	4
Elevated Triglycerides	17	16	3*	2

#Confirmed as HIV positive using VITROS HIV 1+2, an FDA approved HIV antibody differentiation assay and a licensed HIV nucleic acid test.

*Negative on an approved HIV antibody differentiation assay and not detected on a licensed HIV nucleic acid test.

In these samples of potentially cross-reacting substances and medical conditions unrelated to HIV infection, 20 were reactive with the VITROS HIV Combo test and 19 of the 20 were confirmed HIV positive. One sample with high triglycerides was negative in an HIV antibody differentiation assay and HIV-1 RNA was not detected using nucleic acid testing. This sample is considered a VITROS HIV Combo false reactive. This sample had triglyceride concentration of (b) (4) mg/dL; eight additional samples with higher triglyceride concentrations were tested and generated negative results in VITROS HIV Combo. This suggests that the reactivity in VITROS HIV Combo test is not caused by cross reactivity with high triglyceride concentrations, however, this false reactive result shall be included in product labeling. All other samples tested were nonreactive in the VITROS HIV Combo test indicating no cross-reactivity.

f. Potentially Cross Reacting Microbiological Studies

The potential for bacterial contamination to affect the performance of the VITROS HIV Combo test was further evaluated by testing samples spiked with *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* at the concentration of (b) (4). HIV negative and spiked HIV reactive samples (anti-HIV-1, anti-HIV-2, anti-HIV-1 Group O, and HIV p24 antigen) were tested. Of the samples tested, none of the HIV negative samples were found to be false reactive and none of the HIV spiked samples were found to be false negative in the VITROS HIV Combo test. These studies demonstrated that samples with bacterial contamination at the levels tested did not interfere with the performance of the VITROS HIV Combo test.

g. Other Potentially Interfering Substances

The VITROS HIV Combo test was evaluated for interference consistent with CLSI document EP07. Of the compounds tested, none were found to interfere with the clinical interpretation of the test in negative and weakly reactive samples at the concentrations indicated in the Table 4.

Table 4: Concentrations of Potentially Interfering Substances Tested

Test Substance	Maximum Level Tested	
Bilirubin (conjugated)	30 mg/dL	0.386 mmol/L

Bilirubin (unconjugated)	30 mg/dL	0.513 mmol/L
Biotin	20 ng/mL	82.0 nmol/L
Hemoglobin	500 mg/dL	0.078 mmol/L
Cholesterol	300 mg/dL	77.7 mmol/L
HAMA	263 ng/mL	NA
IgG	2380 mg/dL	23.80 g/L
RF	3020 IU/mL	NA
Total Protein	10.9 g/dL	109 g/L
Triglycerides	1250 mg/dL	14.13 mmol/L
Intralipid	850 mg/dL	NA

NA = alternate units not provided

h. Reproducibility

The reproducibility study was performed at three external sites using three reagent lots. Three replicates each of a fourteen-member panel were tested twice per day on six different days. The between run, between day, between site, between lot, and total precision estimates (CV (%)) were derived from a variance component analysis, and are shown in Table 5.

Table 5: VITROS HIV Combo test Precision Estimates

Panel Member	**Grand Mean**	**Within Run**		**Between-Run/Operator**		**Between-Day**		**Within-Laboratory (Total)***		**Between Site****		**Between Lot*****		**Overall********		**No. Obs.**
	(S/C)	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	
Negative	0.12	0.050	N/A[+]	0.000	N/A[+]	0.026	N/A[+]	0.057	N/A[+]	0.019	N/A[+]	0.025	N/A[+]	0.066	N/A[+]	324
Anti-HIV-1	0.76	0.050	6.6	0.017	2.2	0.043	5.7	0.068	8.9	0.000	0.0	0.074	9.8	0.112	14.7	324
Anti-HIV-1	1.28	0.080	6.2	0.020	1.6	0.052	4.1	0.098	7.6	0.000	0.0	0.083	6.5	0.147	11.5	324
Anti-HIV-1	2.70	0.135	5.0	0.032	1.2	0.076	2.8	0.158	5.8	0.000	0.0	0.154	5.7	0.246	9.1	324
Anti-HIV-2	0.95	0.062	6.6	0.015	1.6	0.051	5.3	0.081	8.6	0.018	1.9	0.127	13.5	0.158	16.7	324
Anti-HIV-2[a]	1.30	0.081	6.2	0.020	1.5	0.056	4.3	0.100	7.7	0.012	0.9	0.153	11.7	0.191	14.7	323
Anti-HIV-2	2.94	0.149	5.1	0.022	0.7	0.081	2.8	0.171	5.8	0.000	0.0	0.206	7.0	0.297	10.1	324
Anti-HIV-1 Group O	1.14	0.069	6.1	0.019	1.7	0.050	4.4	0.087	7.7	0.000	0.0	0.173	15.3	0.201	17.7	324
Anti-HIV-1 Group O	1.43	0.097	6.8	0.032	2.3	0.064	4.5	0.121	8.5	0.000	0.0	0.187	13.1	0.227	15.9	324
Anti-HIV-1 Group O	2.93	0.167	5.7	0.060	2.0	0.077	2.6	0.193	6.6	0.000	0.0	0.253	8.6	0.343	11.7	324
HIV p24 Ag[b]	0.90	0.082	9.1	0.072	8.0	0.051	5.7	0.121	13.4	0.052	5.8	0.055	6.1	0.161	17.8	323

HIV p24 Ag	1.58	0.113	7.2	0.096	6.1	0.054	3.4	0.158	10.0	0.050	3.2	0.135	8.6	0.243	15.4	324
HIV p24 Ag	3.63	0.216	6.0	0.244	6.7	0.080	2.2	0.335	9.2	0.085	2.3	0.442	12.2	0.609	16.8	324
Negative	0.13	0.030	N/A[+]	0.004	N/A[+]	0.026	N/A[+]	0.040	N/A[+]	0.017	N/A[+]	0.024	N/A[+]	0.051	N/A[+]	324

N/A = Not applicable

[*] Within-Laboratory (Total) variability contains the within-run, between-run, and between-day variance components.

[**] 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, technologist and day.

[+] % CV are not meaningful when S/C approaches zero

[a] One replicate that had a S/C ratio of 12.4 was excluded from the analysis. When this replicate was included, the mean S/C ratio was 1.34, the Within-Run CV (%) was 46.6, the Between Run/operator CV (%) was 0.0, the Between Day CV (%) was 3.8, the Within Laboratory CV (%) 46.8, the Between Site CV (%) was 0.0, the Between Lot CV (%) was 8.8, and the Overall CV (%) was 48.1.

[b] One replicate that had a S/C ratio of 5.44 was excluded from the analysis. When this replicate was included, the mean S/C ratio was 0.92, the Within-Run CV (%) was 28.8, the Between Run/operator CV (%) was 6.9, the Between Day CV (%) was 6.9, the Within Laboratory CV (%) was 30.5, the Between Site CV (%) was 5.8, the Between Lot CV (%) was 6.6, and the Overall CV (%) was 32.6.

XIV. Summary of Clinical Studies

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

Sensitivity in Individuals Infected with HIV-1

Samples from 973 adults infected with HIV-1 residing in the U.S. were tested with the VITROS HIV Combo test and a FDA- approved HIV Ag/Ab Combo test. All samples were positive for anti-HIV-1. The results for the VITROS HIV Combo test and a FDA approved comparator assays when testing U.S. HIV-1 positive samples are presented in the Table 6.

Table 6: Performance of the VITROS HIV Combo test when testing U.S. HIV-1 Antibody Positive Samples

Population Description	N	FDA-Approved HIV Ag/Ab Combo Test		VITROS HIV Combo test	
		Negative	Reactive	Negative	Reactive
U.S. Adults	973	0	973	0	973

The sensitivity of the VITROS HIV Combo when testing samples collected from HIV-1 infected individuals from the United States is 100% (973/973; 95% CI = 99.62% to 100%).

Samples from 459 subjects infected with HIV-1 and residing in countries other than the United States (i.e., countries from Africa, Asia and Europe) were tested with the VITROS HIV Combo test and a FDA-approved HIV Ag/Ab Combo test. The distribution of VITROS HIV Combo results for these samples is presented in the Table7.

Table 7: Performance of the VITROS HIV Combo Test in International HIV-1 Antibody Positive Samples

Population Description	N	FDA-Approved HIV Ag/Ab Combo Test		VITROS HIV Combo test	
		Negative	Reactive	Negative	Reactive
HIV-1 Positive International	459	0	459	0	459

All samples from 459 HIV-1 infected international samples that were known positive for anti-HIV-1 antibody tested reactive with the VITROS HIV Combo test. The sensitivity of the VITROS HIV Combo test in these samples is 100% (459/459; 95% CI = 99.20% to100%).

Out of these 459 anti-HIV-1 positive international samples, information on the HIV-1 group and subtypes of HIV-1 group M was available for 413 samples. The performance of VITROS HIV Combo test for different HIV types and subtypes is presented in the Table 8.

Table 8: Performance of the VITROS HIV Combo Test When Testing HIV-1 Antibody Positive Samples by Group and Subtype

Group	Subtype/CRF	FDA-Approved HIV Ag/Ab Combo Test Number Reactive/Number Tested	VITROS HIV Combo test Number Reactive/Number Tested
O	N/A	30/30	30/30
M	A	41/41	41/41
	A1	12/12	12/12

A2	2/2	2/2
AE	1/1	1/1
AG	13/13	13/13
B	2/2	2/2
C	49/49	49/49
CRF01	4/4	4/4
CRF01/A1	1/1	1/1
CRF01/AE	7/7	7/7
CRF01/CRF15	1/1	1/1
CRF02/AG	106/106	106/106
CRF02/G	2/2	2/2
CRF05	1/1	1/1
CRF06	5/5	5/5
CRF07	1/1	1/1
CRF09	2/2	2/2
CRF09/K	1/1	1/1
CRF11	2/2	2/2
CRF13	6/6	6/6
D	24/24	24/24
F	10/10	10/10
F1	3/3	3/3
F2	12/12	12/12
G	39/39	39/39
H	18/18	18/18
H/A1	1/1	1/1
H/U	1/1	1/1
J	7/7	7/7
K	8/8	8/8
U	1/1	1/1
Totals	413/413	413/413

This study demonstrated that the VITROS HIV Combo test was reactive for different HIV-1 types and subtypes tested.

An additional 41 HIV-1 Group O samples had sufficient volume only for VITROS HIV Combo testing, and 40/41 (97.56%) tested reactive. The one sample with a negative result did not have sufficient volume for supplemental testing and was therefore classified as HIV positive based on the information provided in the certificate of analysis.

Sensitivity in Individuals Infected with HIV-2

Sensitivity of the VITROS HIV Combo test was also determined using samples collected from 229 individuals from West Africa infected with HIV-2, and positive only for anti-HIV-2 antibody. Testing results are summarized in Table 9.

Table 9: Performance of the VITROS HIV Combo Test When Testing Individuals Infected with HIV-2 only

Population Description	N	FDA-Approved HIV Ag/Ab Combo Test		VITROS HIV Combo test	
		Negative	Reactive	Negative	Reactive
HIV-2 Positive	229	0	229	0	229

All 229 samples from individuals infected with HIV-2 only that were positive for anti-HIV-2 antibody, tested reactive with the VITROS HIV Combo test. The sensitivity of the VITROS HIV Combo test in these anti-HIV-2 antibody positive samples is 100% (229/229; 95% CI = 98.40% to 100%).

Sensitivity for HIV-1 p24 Antigen

A total of 52 samples that were positive for HIV-1 p24 antigen were tested with the VITROS HIV Combo test. Testing results are shown in Table 10 below.

Table 10: Performance of the VITROS HIV Combo Test When Testing HIV-1 p24 Antigen Positive, Antibody Negative Samples

Description (Population)	N	FDA-Approved HIV Ag/Ab Combo Test		VITROS HIV Combo test	
		Negative	Reactive	Negative	Reactive
HIV Antigen Positive Samples	16	1*	15	1*	15
Seroconversion Panel Samples	29	2**	27	1***	28
p24 Antigen Mixed Titer Panel Samples****	7	NT	7 (NT)	0	7
Total	52	3	49	2	50

* Supplemental test was positive only for HIV-1 RNA (NAT). The certificate of analysis indicated that the concentration of p24 antigen was near the limit of detection of both tests.

** Two seroconversion panel samples were negative with the FDA-approved HIV Ag/Ab Combo test and were reactive with the VITROS HIV Combo test.

*** One seroconversion panel sample was negative with the VITROS HIV Combo test and was reactive with the FDA-approved HIV Ag/Ab Combo test.

**** Samples from the p24 Antigen Mixed Titer Panel were not tested (NT) with the FDA-Approved HIV Ag/Ab Combo Test in this study. These well characterized samples were noted as reactive with the FDA approved HIV Ag/Ab combo test in the panel data sheet.

The sensitivity of the VITROS HIV Combo test in HIV-1 p24 Antigen Positive, Antibody Negative Samples was 96.15% (50/52; 95% CI = 86.79% to 99.53%).

High Risk Population in the United States

Samples from 1004 subjects at high risk for HIV infection were tested with the VITROS HIV Combo test and a FDA-approved, HIV Ag/Ab Combo test (with supplemental testing as required). All subjects were at risk due to lifestyle, behavior, occupation, and known exposure events. These subjects were from California, Georgia and Florida. Seventy-four (7.4%) of the 1004 samples tested reactive with the VITROS HIV Combo test, and out of these, 71 tested confirmed positive. Data are shown in Table 11.

Table 11: Performance of VITROS HIV Combo Test with HIV Status in the U.S. Adult High Risk Population

Population Description	Number Tested	FDA-Approved HIV Ag/Ab Combo Test			VITROS HIV Combo test			Supplemental Positive
		Negative	IR	Reactive	Negative	IR	Reactive	
Adult	1004	932	72	72	930	74	74	71

IR = Initially Reactive

In this study, the sensitivity of the VITROS HIV Combo test in the high risk populations is 100% (71/71 with a 95% CI of 94.94% to 100%). The specificity of the VITROS HIV Combo test in the high risk populations was 99.68% (930/933 in this study with a 95% CI of 99.06% to 99.93%).

Population at High Risk for Infection with HIV-2

The performance of the VITROS HIV Combo test was evaluated among individuals at high risk for HIV-2 infection due to residence in an HIV-2 endemic area. The 522 subjects residing in HIV-2 endemic areas of West Africa were prospectively enrolled in this study. Testing results are presented in Table 12.

Table 12: Performance of the VITROS HIV Combo test in Individuals at High Risk for HIV-2 Infection

Population Description	Number Tested	FDA-Approved HIV Ag/Ab ComboTest			VITROS HIV Combo test			Supplemental Positive
		NR	IR	R	NR	IR	R	
West Africa	522	507	15	15	502	20	20	14

NR = non-reactive (negative); IR = initially reactive; R = reactive

The VITROS HIV Combo test was reactive in 20 samples; 14 of these were confirmed positive by a supplemental test. The FDA-approved HIV Ag/Ab Combo test was reactive in 15 samples; 14 of these were confirmed positive by a supplemental test. Of the 14 (2.7%) positive supplemental testing results, ten (71.4%) were positive for HIV-1, three (21.4%) were positive

for HIV-2 and one (7.1%) was HIV positive undifferentiated. Five of the 14 (35.7%) supplemental positive samples were from pregnant subjects.

The determined sensitivity of the VITROS HIV Combo test in the HIV-2 high risk population is 100% (14/14 in this study with a 95% CI of 76.84% to 100.00%). The determined specificity of the VITROS HIV Combo test in the high risk populations is 98.82% (502/508, 95% CI = 97.45% to 99.57%) compared with 99.80% (507/508) for the FDA-approved HIV Ag/Ab Combo test.

Pregnant Women Populations

Six hundred thirty-four (634) samples from pregnant women were tested with the VITROS HIV Combo test and a FDA-approved HIV Ag/Ab Combo test (with supplemental testing as required). The samples were from individuals at low risk for HIV infection (N=262), individuals at low risk for HIV infection in the period around labor and delivery (N=60), individuals at high risk for HIV infection (N=263), and individuals positive for HIV infection (N=49).

Specificity in Pregnant Women at Low Risk for infection with HIV

Of the 262 pregnant women at low risk for infection with HIV, 25.6% were in their first trimester, 30.1% were in their second trimester, and 44.3% were in their third trimester. Study subjects were from California, Georgia, and Florida.

None of the samples from these 262 pregnant women tested reactive with the VITROS HIV Combo test. None of the 60 samples from pregnant women at the time of labor and delivery tested reactive with the VITROS HIV Combo test. The results obtained from the 322 samples from pregnant women at low risk for infection with HIV are summarized in Table 13.

Table 13: Performance of the VITROS HIV Combo test in Pregnant Women Populations at Low Risk

Population Description	Number Tested	FDA-Approved HIV Ag/Ab Combo Test			VITROS HIV Combo test			Supplemental Test Positive
		NR	IR	R	NR	IR	R	
Pregnant Women	262	261	1	1	262	0	0	0
Labor & Delivery	60	0	0	0	60	0	0	0
Total	322	321	1	1	322	0	0	0

NR = non-reactive (negative); IR = initially reactive; R = reactive

The specificity of the VITROS HIV Combo test was calculated as the percentage of the HIV negative samples that tested negative with the test. The specificity of the VITROS HIV Combo

test in pregnant women at low risk for infection with HIV is 100%. (322/322; 95% CI = 98.86% to 100%) compared with 99.69% (321/322) for the FDA-approved HIV Ag/Ab Combo test.

Sensitivity in Pregnant Women Infected with HIV-1

Samples from 49 HIV-1 infected pregnant women residing in the U.S. were tested with the VITROS HIV Combo test and a FDA-approved HIV Ag/Ab Combo test. Of the 49 pregnant women, 30.6% were in their first trimester, 36.7% were in their second trimester, and 32.7% were in their third trimester. The distribution of VITROS HIV Combo results among the samples from U.S. pregnant women infected with HIV-1 is presented in Table 14.

Table 14: Performance of the VITROS HIV Combo test in U.S. pregnant women infected with HIV-1 Samples

Population Description	N	FDA-Approved HIV Ag/Ab Combo Test		VITROS HIV Combo test	
		Negative	Reactive	Negative	Reactive
Pregnant Women HIV Positive	49*	0	49	0	49

*All samples were positive for anti-HIV-1 antibody.

The sensitivity of the VITROS HIV Combo test in samples from pregnant women infected with HIV-1 from the U.S. is 100% (49/49; 95% CI = 92.75% to 100%).

Pregnant Women at High Risk for Infection with HIV

Of the 263 pregnant women at high risk for infection with HIV, 34.6% were in their first trimester, 32.7% were in their second trimester, and 32.7% were in their third trimester. Subjects were enrolled in California, Georgia and Florida. Two of the 263 samples were reactive with the VITROS HIV Combo test. The VITROS HIV Combo test results among pregnant women at HIV high risk for infection with HIV are presented in Table 15.

Table 15: Pregnant Women Population at High Risk for infection with HIV

Population Description	Number Tested	FDA-Approved HIV Ag/Ab Combo Test			VITROS HIV Combo test			Supplemental Positive
		NR	IR	R	NR	IR	R	
Pregnant Women	263	262	1	1	261	3	2	1

NR = non-reactive (negative); IR = initially reactive; R = reactive

One (0.4%) of the 263 high risk samples was reactive with the VITROS HIV Combo test and positive by supplemental testing. The one sample testing supplemental positive was also reactive with the FDA-approved HIV Ag/Ab Combo test. In this study the determined

specificity of the VITROS HIV Combo test in the HIV high risk pregnant women is 99.62% (261/262; 95% CI = 97.89% to 99.99%).

In addition to the one confirmed positive sample from the U.S. HIV high risk pregnant women population, an additional five confirmed positive samples from pregnant women were detected in the HIV-2 high risk population.

Pediatric Populations

Four hundred fifteen (415) samples from pediatric subjects were tested with the VITROS HIV Combo test and a FDA-approved HIV Ag/Ab Combo test (with supplemental testing as required). An additional 11 samples only had volume sufficient for VITROS HIV Combo testing. Overall, the samples were from individuals at low risk for HIV infection (N=110), individuals at high risk for HIV infection (N=251), and individuals positive for HIV infection (N=54, plus samples with volume only for VITROS HIV Combo testing, N=11). The pediatric subjects ranged in age from 2 to 20 years.

Sensitivity in Pediatric Subjects Infected with HIV-1

Samples from 54 HIV-1 infected pediatric subjects residing in the U.S. were tested with the VITROS HIV Combo test and a FDA-approved HIV Ag/Ab Combo test. Data are shown in Table 16.

Table 16: Performance of the VITROS HIV Combo test in Samples from Pediatric Subjects infected with HIV-1

Population Description	N	FDA-Approved HIV Ag/Ab Combo Test		VITROS HIV Combo test	
		Negative	Reactive	Negative	Reactive
HIV Positive Pediatric	54	1*	53	1**	53

* One sample tested negative with the FDA-approved HIV Ag/Ab Combo test and tested reactive with the VITROS HIV Combo test. Sample volume was insufficient for supplemental testing. The sample was assigned an HIV status of positive based on the certificate of analysis.

** This sample tested negative with the VITROS HIV Combo test and tested reactive the FDA-approved HIV Ag/Ab Combo. Sample volume was insufficient for supplemental testing. The sample was assigned an HIV status of positive based on the certificate of analysis.

All but one of the 54 samples tested reactive with the VITROS HIV Combo test. The sensitivity of the VITROS HIV Combo test in U.S. pediatric subjects infected with HIV-1 is 98.15% (53/54; 95% CI = 90.11% to 99.95%).

Eleven (11) additional U.S. pediatric samples positive for HIV-1 infection by certificate of analysis only had sufficient volume for VITROS HIV Combo testing and 11/11 (100.00%) tested reactive.

Specificity in Pediatric Subjects at Low Risk for infection with HIV

Samples from 110 pediatric subjects from Florida at low risk for HIV infection were tested. Of the 110 pediatric low risk subjects, 59.1% were male and 40.9% were female, ranging in age from 2‑20 years. One (0.9%) of the 110 samples was reactive with the VITROS HIV Combo test. The VITROS HIV Combo results for the low risk pediatric subjects are presented in Table 17.

Table 17: Performance of the VITROS HIV Combo test and FDA-Approved HIV Ag/Ab Combo Test in Pediatric Low Risk Populations

Population Description	N	FDA-Approved HIV Ag/Ab Combo Test			VITROS HIV Combo test			Supplemental Positive
		NR	IR	R	N	IR	R	
Pediatric	110	110	0	0	109	1	1	0

NR = non-reactive (negative); IR = initially reactive; R = reactive

In this study, the specificity of the VITROS HIV Combo test in the pediatric low risk population is 99.09% (109/110, 95% exact confidence interval CI = 95.04% to 99.98%).

Pediatric Subjects at High Risk for Infection with HIV

Of the 251 enrolled pediatric high risk subjects, 46.2% were male and 53.8% were female. These subjects were from Georgia and Puerto Rico. Seven (2.8%) of the 251 samples tested reactive with the VITROS HIV Combo test. Data are shown in Table 18.

Table 18: Performance of the VITROS HIV Combo Test in the U.S. Pediatric High Risk Population

Population Description	Number Tested	FDA-Approved HIV Ag/Ab Combo Test			VITROS HIV Combo test			Supplemental Positive
		NR	IR	R	NR	IR	R	
Pediatric	251	248	3	3	244	8	7	3

NR = non-reactive (negative); IR = initially reactive; R = reactive

Three (1.2%) of the 251 samples tested reactive with the VITROS HIV Combo test, and positive by supplemental testing. All three of those samples tested positive on supplemental test were reactive with the FDA-approved HIV Ag/Ab Combo test.

An additional four samples that tested reactive with the VITROS HIV Combo test tested negative on the supplemental test. The specificity of the VITROS HIV Combo test in the pediatric high risk population is 98.39% (244/248, 95% CI =95.92% to 99.56%).

XV. Instrument and Software

The VITROS HIV Combo test operates on VITROS 3600 Immunodiagnostic System (VITROS System).

The VITROS System has been previously reviewed and approved under BP050051/S018 for the use of VITROS Anti-HIV 1+2 Assay on the System. The VITROS System has also been cleared under a Pre-Market Notification (510k) K083178.

The Sponsor states that the VITROS HIV Combo test leverages existing VITROS 3600 Immunodiagnostic System functionality, therefore no changes have been made to the software or hardware of the VITROS System.

Since the VITROS System has been reviewed in submissions noted above, the software/instrumentation review primarily focused on the Assay Data Disk (ADD) which contains the source data used to define the parameters for assays and calibrations in System software.

The points listed below are a Summary of information provided by the Sponsor in the Original PMA BP160122 and its subsequent Amendments.

- ***Versioning:*** The Sponsor states that the VITROS HIV Combo test operates on the VITROS 3600 Immunodiagnostic System Software version 3.3.1. The VITROS System utilizes QNX Operating System (OS) version 6.3.0 SP2. Each Assay Data Disk (ADD) has a unique Data Release Version number which is updated based on the assay, reagent lots, calibrators, etc.

- ***Device Description:*** The VITROS 3600 Immunodiagnostic System allows for the determination of analytes in human samples (for example, serum and plasma). Assays on the VITROS System employ a chemiluminescence detection reaction. The analyzer is fully automated with a refrigerated, on board assay storage system. The VITROS System also provides menu driven software, which can be accessed from a touch screen monitor. The VITROS System has a throughput of up to (b) (4) assays per hour.
  The Assay Data Disk (ADD) is the source data used to define the parameters for assays and calibrations in the System Software. The System Software uses this data to perform assay tests, calibrations, controls, and corresponding configurations. All reagent/calibrator lot-specific information is downloaded via the ADD. This is a standard CD (Compact Disk) that is issued periodically to customers. This data CD contains all of the analyte protocol definition information (analyte name, operational characteristics, etc.) as well as the lot specific information for each analyte (lot number, expiration date, calibrator concentrations, calibrator ranges, quality check parameters, etc.). This CD is loaded onto the system by the operator, and the information is transferred to the internal hard disk drive so that it can be accessed by the on-board computer during on-analyzer calibration.

- ***Risk Analysis:*** A System level hazard analysis and risk assessment has been conducted by the Sponsor to establish that all significant system hazards have been adequately addressed when the VITROS 3600 Immunodiagnostic System is used with VITROS HIV Combo test. The Sponsor has listed numerous risks and their corresponding mitigations. Some of the identified risks include: "Imprecise Incubation Temperature,", "Sample Volume Accuracy not maintained," "Cross-contamination between MicroWells," etc. The Sponsor has provided detailed cross-references to requirements, test procedures and test cases that support verification of the features/processes which serve as safeguard to these risks.

- ***Testing:*** The Sponsor has provided a description of the Validation and Verification activities conducted on the VITROS System. The testing activities include Intended Use Testing, Assay Verification, Instrument Verification & Validation, Software Verification & Validation. The Sponsor has performed internal validation which focused on software usability and hardware-software interactions in a simulated customer environment. Features tested include: Automation Track Interface, customer testing profiles/panels, reagent and consumable inventory management, Assay calibration and quality control, fluid types tested, preventive maintenance. The Sponsor has also performed validation of the System by utilizing it in non-clinical studies, as well as clinical studies at external clinical sites.

- ***Unresolved Anomalies:*** The Sponsor has provided a list of Unresolved Anomalies (UA) in the original documentation. In subsequent Amendments, the Sponsor has provided additional information by discussing the anomalies they are monitoring, the likelihood of occurrence and their mitigation plan. The Sponsor has stated that they have corrected the issues which had major/critical severity associated with them. According to the Sponsor, they will monitor existing anomalies and will fix moderate/low severity defects in the next release version of the System Software.

Development Management: The Sponsor has provided a summary of their software development life cycle plan, describing the processes that have been put into place to manage the various software development life cycle activities, including a summary of the configuration management and maintenance activities.

The conclusion of the software review is that the information provided is acceptable.

XVI. Facility Inspection

A pre-approval inspection of the Ortho-Clinical Diagnostics manufacturing facility located in Felindre Meadows, Pencoed, Bridgend, South Wales, United Kingdom, was conducted by FDA on September 18-21, 2017. Following the inspection of validation processes and the manufacturing controls, the inspection was classified as No Action Indicated (NAI). Reviewers in the Office of Compliance and Biologics Quality (OCBQ)/Division of Manufacturing and Product Quality (DMPQ) recommended approval of this PMA.

XVII. Conclusions Drawn from the Studies

Risk/Benefit Analysis

The VITROS HIV Combo test provides useful information to the patient and healthcare provider on the HIV status of an individual, which can serve as an aid in the diagnosis of infection with HIV-1 or HIV-2.

Risks associated with VITROS HIV Combo test relate primarily to its rate of false negative and false positive results. Performance studies have demonstrated that VITROS HIV Combo test has a high level of sensitivity and specificity; (i.e., the rate of false reactive or false nonreactive results with VITROS HIV Combo Assay Combo is very small). Overall, the information provided by the sponsor indicates that the benefits of VITROS HIV Combo test outweigh the risks associated with its use.

Safety and Effectiveness

Multi-center clinical studies were conducted in the U.S. The VITROS HIV Combo test demonstrated clinical sensitivity and specificity comparable to a FDA approved HIV Combo assay. Results from the clinical studies indicate that the VITROS HIV Combo test can be used safely and effectively for the qualitative in vitro detection of HIV-1 p24 antigen, HIV-1 antibody, HIV-2 antibody and HIV-1 Group O antibody in human specimens (serum and plasma). The specimens with reactive test results must be further tested by the currently recommended algorithm for confirming HIV infection. Confirmatory test results and counseling are considered as an important part of testing for HIV infection. A negative test result at any point does not preclude the possibility of exposure to or infection with HIV. Negative results can occur if the quantity of marker present in the specimen is below the detection limit of the assay, or if the marker is not present during the stage of disease in which a specimen is collected.

The safety and effectiveness of the VITROS HIV Combo test has been demonstrated in both clinical and non-clinical studies. The benefit to individuals tested by this assay outweighs potential adverse events or risks to the patient or user due to assay malfunction or operator error.