VITROS Immunodiagnostic Products

Intended for Use in the United States

INSTRUCTIONS FOR USE HIV c

VITROS Immunodiagnostic Products HIV Combo Reagent Pack	REF	684 2781
VITROS Immunodiagnostic Products HIV Combo Calibrator	REF	684 2782

Rx ONLY

Intended Use

For in vitro diagnostic use only.

VITROS Immunodiagnostic Products HIV Combo Reagent Pack

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 and the VITROS 5600/XT 7600 Integrated Systems.

A reactive test 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 test, 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 test is not intended for use in screening blood or plasma donors. The effectiveness of the VITROS HIV Combo test for use in routine screening of blood, plasma, cell or tissue donors has not been established. However, this test can be used as a blood donor screening test 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

VITROS Immunodiagnostic Products HIV Combo Calibrator is for use in the calibration of the VITROS 3600 Immunodiagnostic System and the VITROS 5600/XT 7600 Integrated Systems with the VITROS HIV Combo Reagent Pack.

Summary and Explanation of the Test

Acquired Immunodeficiency Syndrome (AIDS) is caused by two types of human immunodeficiency viruses designated HIV-1 and HIV-2. The VITROS HIV Combo test uses 3 recombinant antigens derived from HIV-1 envelope (env13), HIV-1 group O envelope (env70-3) and HIV-2 envelope (env31). These antigens detect antibodies to HIV-1 and antibodies to HIV-2 in the same test. The use of these recombinant antigens improves test specificity by avoiding non-specific 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 p24 antigen to enable detection of HIV p24 antigen that may be present prior to the onset of antibody response enabling earlier diagnosis of HIV-1 infection.

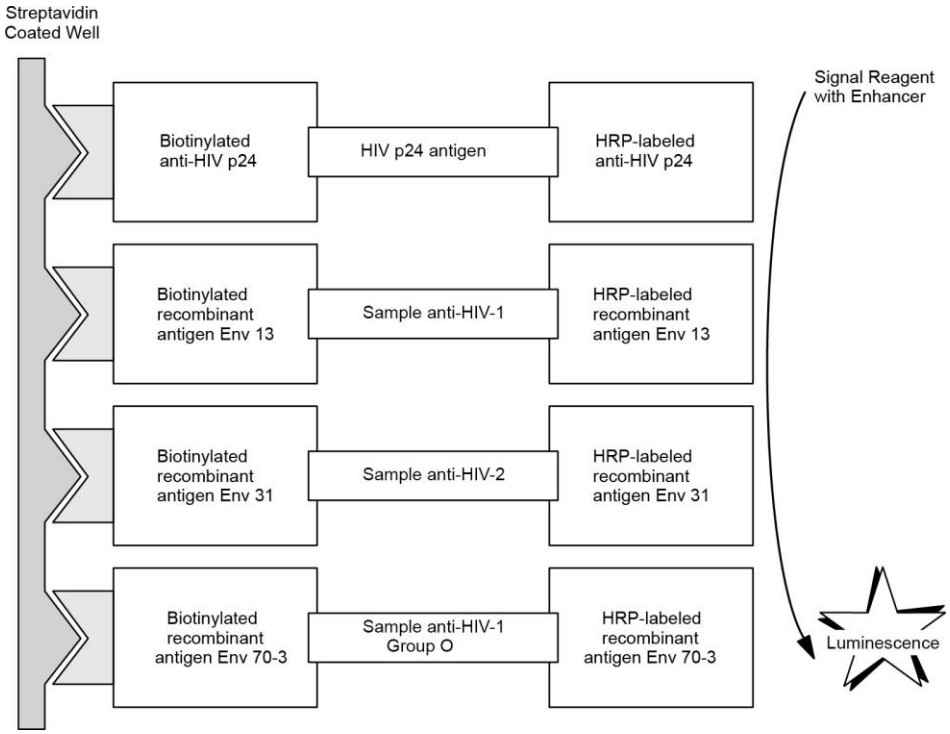
Principles of the Procedure

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 and the VITROS 5600/XT 7600 Integrated Systems using Intellicheck® Technology. An immunometric technique is used; this involves a two stage reaction. In the first stage HIV antibody or antigen present in the sample binds with 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. [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 system. The amount of HRP conjugate bound is indicative of the amount of HIV antibody and/or p24 antigen present.
The key features of the VITROS HIV Combo test method are summarized in the following table.

Test Type	System	Incubation Time	Time to first result	Test Temperature	Reaction Sample Volume
Immunometric	3600, 5600, XT 7600	37 minutes	48 minutes	37 ° C	80 µL

Reaction scheme



Warnings and Precautions

For in vitro diagnostic use only.
Read the Instructions for Use completely before using this test. Follow the instructions carefully as not doing so may result
in inaccurate test results.

WARNING: Potentially Infectious Material

Treat as if capable of transmitting infection.

Use caution when handling material of human origin. Consider all samples 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). [2]

The VITROS HIV Combo Calibrator contains:

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

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.

WARNING: Contains ProClin 300 (CAS 55965–84–9) [3]

The VITROS HIV Combo Reagent Pack contains 1.0% ProClin 300. H317: May cause an allergic skin reaction. P280: Wear protective gloves/protective clothing/eye protection/face protection. P302 + P352: IF ON SKIN: Wash with plenty of soap and water. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P363: Wash contaminated clothing before reuse.

WARNING

WARNING: Contains ProClin 950 (CAS 2682-20-4) [3]

The VITROS HIV Combo Calibrator contains 1.0% ProClin 950. H315: Causes skin irritation. H317: May cause an allergic skin reaction. H319: Causes serious eye irritation. P280: Wear protective gloves/protective clothing/eye protection/ face protection. P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P337 + P313: If eye irritation persists: Get medical advice/attention.
P321: Specific treatment (see Section 4, First aid measures, in Safety Data Sheet).

Refer to www.Orthoclinicaldiagnostics.com for the Safety Data Sheets and for Ortho contact information.

WARNING

Reagents

Reagent Pack Contents

1 reagent pack containing:

- 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

Reagent Pack Handling

- The reagent pack is supplied ready for use.
- The reagent pack contains homogeneous liquid reagents that do not require shaking or mixing prior to loading on the system.
- As with all immunoassay protein-based solutions, inappropriate handling of the reagent pack can cause foam to occur on the surface of the reagent. Avoid agitation, which may cause foaming or the formation of bubbles.
  - If reagent packs are dropped or agitated, small levels of fine foam could be generated that may not be detected by the system.
  - Reagent packs containing fine foam that is not detected by the system may show a negative bias.
  - If you must use a dropped or agitated reagent pack before it has been allowed to settle, you should verify performance by running quality control samples in duplicate after loading the pack on the system.

Reagent Pack Storage and Preparation

Reagent	Storage Condition	Stability
Unopened	Refrigerated 2–8 ° C (36–46 ° F)	expiration date
Opened	On system System turned on	≤12 weeks
Opened	Refrigerated 2–8 ° C (36–46 ° F)	≤12 weeks

- The VITROS HIV Combo Reagent Pack is suitable for use until the expiration date on the carton when stored and handled as specified. Do not use beyond the expiration date.
- Do not freeze unopened reagent packs.
- Load reagent packs directly from refrigerated storage to minimize condensation.
- Store opened refrigerated reagent packs in a sealed reagent pack storage box that contains dry desiccant.

Calibrator Contents

- 1 VITROS HIV Combo Calibrator (anti-HIV-1 positive human plasma in anti-HIV 1+2 negative human plasma, 2.0 mL) with antimicrobial agent
- 8 calibrator bar code labels
- Lot Calibration Card
- Protocol Card - Major Protocol Version 2

Calibrator Handling

- Use only with reagent packs of the same lot number. Mix thoroughly by inversion and bring to 15–30 ° C (59–86 ° F) before use. Each pack contains sufficient volume for a minimum of 6 determinations of each calibrator.
- Handle calibrators in stoppered containers to avoid contamination and evaporation. To avoid evaporation, limit the amount of time calibrators are on the system. Refer to the operating instructions for your system.
- Return to 2–8 ° C (36–46 ° F) as soon as possible after use, or load only sufficient volume for a single determination.

Calibrator Storage and Preparation

Calibrator	Storage Condition	Stability
Unopened	Refrigerated 2–8 ° C (36–46 ° F)	expiration date
Opened	Refrigerated 2–8 ° C (36–46 ° F)	≤13 weeks
Opened	Frozen -20 ° C (-4 ° F)	≤13 weeks

- The VITROS HIV Combo Calibrator is supplied ready for use.
- The VITROS HIV Combo Calibrator is suitable for use until the expiration date on the carton when stored and handled as specified. Do not use beyond the expiration date.
- The opened calibrator may be stored frozen (with no more than 1 freeze-thaw cycle).
- The VITROS HIV Combo test uses 80 μL of calibrator for each determination. The VITROS HIV Combo Calibrator may be used directly on the VITROS 3600 Immunodiagnostic System and the VITROS 5600/XT 7600 Integrated Systems. Alternatively, transfer an aliquot of the calibrator into a sample container (taking account of the minimum fill volume of the container), which may be bar coded with the labels provided. For details on minimum fill volume of sample cups or containers, refer to the operating instructions for your system.
- The VITROS HIV Combo Calibrator is automatically processed in duplicate.

Specimen Collection, Preparation and Storage

Patient Preparation

No special patient preparation is necessary.

Specimens Recommended

- Serum
- Serum Separator Tube (SST)
- Plasma Separator Tube (PST)
- Lithium heparin plasma
- Sodium heparin plasma
- Potassium EDTA plasma

Specimens Not Recommended

Do not use turbid specimens. Turbidity in specimens may affect test results.
Do not use specimens collected in any other anticoagulants or any other body fluids.

Special Precautions

IMPORTANT: Certain collection devices have been reported to affect other analytes and tests. [4] Owing to the variety of specimen collection devices available, Ortho Clinical Diagnostics is unable to provide a definitive statement on the performance of its products with these devices. Confirm that your collection devices are compatible with this test.

Specimen Collection and Preparation

- Collect specimens using standard procedures. [5]
- Samples should be thoroughly separated from all cellular material. Failure to do so may lead to an erroneous result.
- Thoroughly mix samples by inversion and bring to 15–30 ° C (59–86 ° F) before use.
- The VITROS HIV Combo test uses 80 µL of sample for each determination. This does not take account of the minimum fill volume of the chosen sample container. For details on minimum fill volume of sample cups or containers, refer to the operating instructions for your system.

Handling and Storage Conditions

- Handle samples in stoppered containers to avoid contamination and evaporation.
- The amount of time samples are on the system prior to analysis should be limited to avoid evaporation. Refer to the operating instructions for your system.
- Return to 2–8 ° C (36–46 ° F) as soon as possible after use, or load sufficient volume for a single determination.
- Serum, lithium heparin plasma and potassium EDTA plasma samples may be stored for up to 24 hours at room temperature (up to 30 ° C [86 ° F]) or 7 days at 2–8 ° C (36–46 ° F).
- SST, PST and sodium heparin plasma samples may be stored for up to 24 hours at room temperature (up to 30 ° C [86 ° F]).
- Samples that will not be tested within the time frames outlined above should be stored at -20 ° C (-4 ° F) and may be subjected to up to five freeze-thaw cycles.

IMPORTANT: Thoroughly mix thawed samples by multiple inversions or by vortex mixing and **bring to** 15–30 ° C (59–86 ° F) before use.

Materials Provided

- VITROS Immunodiagnostic Products HIV Combo Reagent Pack
- VITROS Immunodiagnostic Products HIV Combo Calibrator

Materials Required but Not Provided; Sold Separately

- VITROS Immunodiagnostic Products Signal Reagent
- VITROS Immunodiagnostic Products Universal Wash Reagent
- Quality control materials
- VITROS Immunodiagnostic Products Reagent Pack Storage Box (optional) with desiccant

Operating Instructions

Check the inventory regularly to aid the management of reagents and ensure that sufficient VITROS Signal Reagent, VITROS Universal Wash Reagent and calibrated reagent lots are available for the work planned. When performing panels of tests on a single sample, ensure that the sample volume is sufficient for the tests ordered.

For detailed information refer to the operating instructions for your system. Note: Do not use visibly damaged product.

Default Test Name

The default test name which will appear on patient reports is HIV Combo. The default short name that will appear on the test selection menus and laboratory reports is HIV c. These defaults may be reconfigured, if required. For detailed information refer to the operating instructions for your system.

Calibration

Calibration Procedure

- Calibration is lot specific; the calibrator lot number linked with reagent pack lot number must be used.
- A Master Calibration is established for each new reagent lot by performing multiple tests. This is the process by which a lot-specific parameter [a] which links the signal at the cutoff (cutoff value) to the calibrator signal is determined.

  Cutoff value = (a x Signal of Cal 1)
- Ensure that the Master Calibration for each new reagent lot is available on your system.
- Process calibrator in the same manner as samples. Load sufficient volume of calibrator for the automatic duplicate determination. Calibration need not be programmed if bar code labels are used; calibration will be initiated automatically.
- When the calibrator is processed, the validity of the calibration is assessed against quality parameters which compare the actual signal of the calibrator with the expected signal. If the calibration is acceptable the cutoff value is calculated and stored for use with any reagent pack of that lot.
- The quality of calibration cannot be completely described by a single parameter. The calibration report should be used in conjunction with acceptable control values to determine the validity of the calibration.
- Recalibration is required after a pre-determined calibration interval, i.e., every 28 days, 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 will be coded in the calibration report. For actions to be taken following a failed calibration refer to the operating instructions for your system.

Refer to the operating instructions for your system for detailed instructions on the calibration process.

When to Calibrate

- When the reagent pack and calibrator lot changes.
- Every 28 days.
- After specified service procedures have been performed.
- If quality control results are consistently outside of your acceptable range.

For additional information on when to calibrate, refer to the operating instructions for your system.

Traceability of Calibration

The calibration of the VITROS HIV Combo test is traceable to an in-house reference calibrator which has been value assigned to optimize clinical sensitivity and specificity.

Calibration Model

Results are calculated as a normalized signal, relative to a cutoff value. During the calibration process a lot-specific parameter is used to determine a valid stored cutoff value for the VITROS 3600 Immunodiagnostic System and the VITROS 5600/XT 7600 Integrated Systems.

Quality Control

Quality Control Material Selection

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 and the VITROS 5600/XT 7600 Integrated Systems.

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 methods if they contain high concentrations of preservatives, stabilizers, or 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.

Quality Control Procedure Recommendations

- Good laboratory practice requires that controls be processed to verify the performance of the test.
- Choose control levels that check the clinically relevant concentrations.
- To verify system performance, analyze control materials:
  - After calibration
  - According to local regulations or at least once each day that the test is being performed
  - After specified service procedures are performed

If quality control procedures within your laboratory require more frequent use of controls, follow those procedures.

- Analyze quality control materials in the same manner as patient specimens.
- If control results fall outside your acceptable range, investigate the cause before deciding whether to report patient results.
- Refer to published guidelines for general quality control recommendations.[6]

For more detailed information, refer to the operating instructions for your system.

Quality Control Material Preparation and Storage

Refer to the manufacturer's product literature for preparation, storage, and stability information.

Results

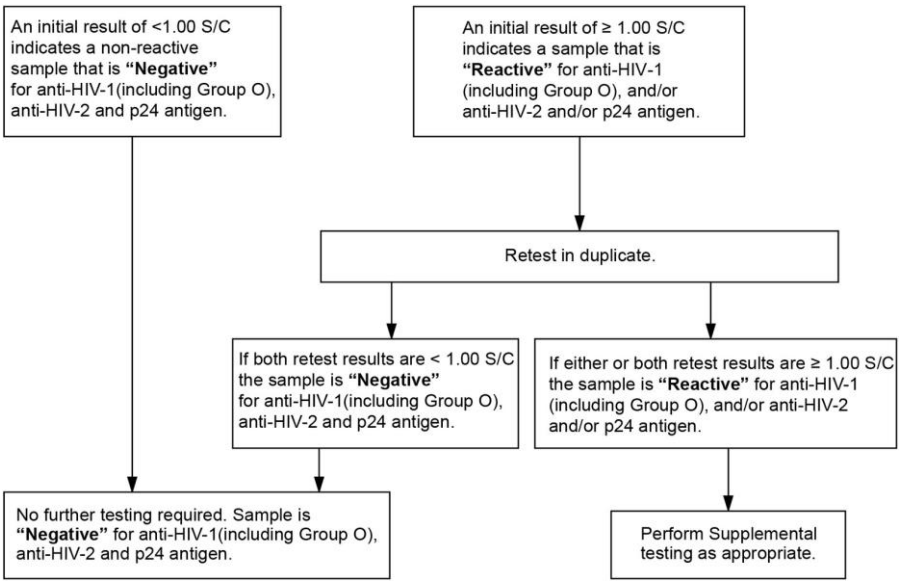
Results are automatically calculated by the VITROS 3600 Immunodiagnostic System and the VITROS 5600/XT 7600 Integrated Systems.

Result Calculation

Results are calculated as a normalized signal, relative to a cutoff value. During the calibration process, a lot-specific parameter is used to determine a valid stored cutoff value for the VITROS 3600 Immunodiagnostic System and the VITROS 5600/XT 7600 Integrated Systems.

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

Caution:	This test will not report HIV c results associated with VITROS 3600 Immunodiagnostic System and VITROS 5600/XT 7600 Integrated Systems 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).



Interpretation of Results

Patient sample results will be displayed with a "Negative" or "Reactive" label.

Initial Test Result (S/C)	Displayed Result	Retest Requirement
<1.00	Negative	No retest required
≥1.00	Reactive	Retest in duplicate

A sample initially found "Reactive" should be retested in duplicate to verify its status. If both repeat results are <1.00 S/C, the sample should be considered negative. If either or both repeat results are ≥1.00 S/C, the sample should be considered reactive and tested by supplemental tests to confirm the results. A repeatedly "Reactive" sample, confirmed by supplemental tests must be considered positive for anti-HIV-1 (including Group O), and/or anti-HIV-2 and/or p24 antigen.

The following table summarizes the final interpretation of results obtained with the VITROS HIV Combo test on the VITROS 3600 Immunodiagnostic System and the VITROS 5600/XT 7600 Integrated Systems.

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), 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, especially in subjects at high risk for infection with HIV 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 counselling, medical intervention and possibly additional testing to decide

whether a diagnosis of infection with HIV is accurate.

- 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 another sample.
- The magnitude of a VITROS HIV Combo test result cannot be correlated to an endpoint titer.

Limitations of the Procedure

Known Interferences

The VITROS HIV Combo test was evaluated for interference consistent with CLSI document EP7. [7] Commonly encountered substances were tested on two lots of reagents. Of the compounds tested, none was found to interfere with the clinical interpretation of the test.

Refer to “Substances that do not Interfere” for a list of compounds tested that did not show interference.

Other Limitations

- 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. [8] 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.
- Certain drugs and clinical conditions are known to alter antibody concentrations in vivo. For additional information, refer to one of the published summaries. [9,10]
- Any testing material including quality control material preserved in azide should not be used.
- This test may not give reactive test results in all infected individuals, therefore, a negative test result 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.
- Use of this test with specimen types other than those specifically approved for use with this device may produce inaccurate test results.
- This test should be performed at 15 - 30° C (59 - 86° F). If stored refrigerated, ensure that the samples are brought to the operating temperature before performing testing.

Performance Characteristics

Clinical Performance

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 infection with HIV, or known to be infected with HIV. 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.

The specificity of the VITROS HIV Combo test was evaluated among individuals at low risk for infection with HIV. The sensitivity of the VITROS HIV Combo test was evaluated among samples collected from individuals infected with HIV. Test performance was further evaluated among individuals with signs or symptoms of infection with HIV and among individuals belonging to groups recognized to be at risk for infection with HIV due to lifestyle, behavior, occupation or known exposure events.

Results by Specimen Classification

Samples from subjects at high or low risk for infection with HIV were tested with a commercially available FDA-approved HIV Ag/Ab Combo test, and with the VITROS HIV Combo test at three testing sites. The HIV status of the sample was defined according to the following HIV Ag/Ab Combo test and supplemental testing algorithm.

Population(s)	HIV Ag/Ab Combo Test Result	Supplemental Testing Results		HIV Status
		HIV-1/HIV-2 Antibody Differentiation Immunoassay	HIV-1 Nucleic Acid Test (NAT)	
Populations Infected with HIV	Reactive	Not Required	Not Required	HIV Positive
	Nonreactive	HIV-1 Positive and/or HIV-2 Positive	Not Required	HIV Positive
		HIV-1 Negative or Indeterminate and HIV-2 Negative	Nonreactive	HIV Negative
			Reactive	HIV Positive
Populations at High and Low Risk for Infection with HIV	Nonreactive	Not Required	Not Required	HIV Negative
	Reactive	HIV-1 Negative or Indeterminate and HIV-2 Negative	Nonreactive	HIV Negative
			Reactive	HIV Positive
		HIV-1 Positive and/or HIV-2 Positive	Not Required	HIV Positive

In those instances where the FDA-approved HIV Ag/Ab Combo test was negative for populations at high and low risk for infection with HIV but the VITROS HIV Combo test was reactive, or where the FDA-approved HIV Ag/Ab Combo test was reactive in subjects enrolled as positive for infection with HIV but the VITROS HIV Combo test was negative, supplemental testing was performed as indicated in the table above to determine the HIV status of the sample.

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 sensitivity of the VITROS HIV Combo test was calculated as the percentage of HIV positive samples that tested reactive with the test.

Specificity in Individuals at Low Risk for Infection with HIV

Samples from 6003 adult subjects at low risk for infection with HIV were tested with the VITROS HIV Combo test and an FDA-approved HIV Ag/Ab Combo test (with supplemental testing as required). These samples were obtained from first time blood donors (N=1998) and other low risk subjects (annual physicals and pre-operation) (N=4005) in the U.S. for whom HIV testing was likely required as part of their group.

Samples from 6003 adult subjects in groups at low risk for infection with HIV were from Florida, Minnesota, New Mexico and Oklahoma. The VITROS HIV Combo test was reactive in 1.15% (69/6003) of the individuals in this group. The distribution of VITROS HIV Combo test results among adult subjects at low risk for infection with HIV is presented in the following table:

Seroreactivity for the VITROS HIV Combo Test in Adult Populations at Low Risk for Infection with HIV

Population Group	VITROS HIV Combo Test Results				Total
	Reactive		Negative		
	N	Percent	N	Percent	
Annual Physical	60	1.5	3864	98.5	3924
First Time Donor	7	0.4	1991	99.6	1998
Pre-Operation	2	2.5	79	97.5	81
Total	69	1.1	5934	98.9	6003

The results obtained for samples from the 6003 adult subjects at low risk for infection with HIV are summarized in the following table:

Performance of the VITROS HIV Combo Test in Populations at Low 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	Negative	IR	Reactive	
Annual Physical	3924	3866	59	58	3864	63	60	44
First Time Donor	1998	1997	2	1	1991	9	7	0
Pre-Operation	81	81	0	0	79	2	2	0
Total	6003	5944	61	59	5934	74	69	44

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

Forty-four (44) (0.73%) of the 6003 samples from adult subjects at low risk for infection with HIV were reactive with the VITROS HIV Combo test and positive by supplemental testing. All 44 of those samples with positive supplemental testing results were reactive with the FDA-approved HIV Ag/Ab Combo test.

The performance of the VITROS HIV Combo test compared with HIV status in adult populations at low risk for infection with HIV is summarized in the following table:

Agreement of the VITROS HIV Combo Test with HIV Status in Adult Populations at Low Risk for Infection with HIV

VITROS HIV Combo Result	HIV Status		Total
	Positive	Negative	
Reactive	44	25	69
Negative	0	5934	5934
Total	44	5959	6003

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 the adult populations at low risk for infection with HIV was 99.58% in this study (5934/5959 with a 95% confidence interval (CI) of 99.38% to 99.73%).

Sensitivity in Individuals Infected with HIV-1

Samples from 973 adults residing in the U.S., infected with HIV and positive for anti-HIV-1, were tested with the VITROS HIV Combo test and an FDA-approved HIV Ag/Ab Combo test. The VITROS HIV Combo test results among the infected
U.S. subjects positive for HIV-1 are presented in the following table:

Performance of the VITROS HIV Combo Test in Infected U.S. Subjects Positive for HIV-1

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

The sensitivity of the VITROS HIV Combo test among the samples collected from infected U.S. subjects positive for HIV-1 was 100.00% (973/973; 95% CI = 99.62% to 100.00%).

Samples from 459 subjects infected with HIV and residing outside of the U.S. (i.e., Africa, Asia and Europe) were

tested with the VITROS HIV Combo test and an FDA-approved HIV Ag/Ab Combo test. The VITROS HIV Combo test results among these samples are presented in the following table:

Performance of the VITROS HIV Combo Test in Infected International Subjects Positive for HIV-1

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

All samples from 459 infected International subjects that were positive for anti-HIV-1 tested reactive with the VITROS HIV Combo test. The sensitivity of the VITROS HIV Combo test in these samples was 100.00% (459/459; 95% CI = 99.20% to 100.00%).

Of these international samples, HIV-1 Group and subtype information was available for 413 samples and test results for these samples using the VITROS HIV Combo test are summarized in the following table:

Performance of the VITROS HIV Combo Test in Infected Subjects Positive for HIV-1 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

Group	Subtype/CRF	FDA-Approved HIV Ag/Ab Combo Test Number Reactive/Number Tested	VITROS HIV Combo Test Number Reactive/Number Tested
	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
Total		413/413	413/413

All 413 samples with HIV-1 Group and subtype information tested reactive with the VITROS HIV Combo test. An additional 41 HIV-1 Group O samples only had sufficient volume 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 among 229 samples collected from individuals from West Africa infected with HIV-2, and positive only for anti-HIV-2. Testing results are summarized in the following table:

Performance of the VITROS HIV Combo Test in Individuals Infected with HIV-2 Alone

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

All 229 samples from individuals infected with HIV-2, that were positive for anti-HIV-2, tested reactive with the VITROS HIV Combo test. The sensitivity of the VITROS HIV Combo test in these anti-HIV-2 positive samples was 100.00% (229/229; 95% CI = 98.40% to 100.00%).

Sensitivity for HIV-1 p24 Antigen

A total of 52 samples positive for HIV-1 p24 antigen were tested with the VITROS HIV Combo test. Testing results are shown in the following table:

Performance of the VITROS HIV Combo Test in 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	0	7****	0	7
Total	52	3	49	2	50

* Supplemental testing was positive only in HIV-1 RNA testing (NAT). The certificate of analysis indicated a p24 antigen level 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 only tested with the VITROS HIV Combo test in the 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%).

Reactivity in Populations at High Risk for Infection with HIV

Reactivity in Populations at High Risk for Infection with HIV-1

Samples from 1004 U.S. subjects at high risk for infection with HIV-1 were tested with the VITROS HIV Combo test and an FDA-approved HIV Ag/Ab Combo test (with supplemental testing as required). All individuals were at risk of infection with HIV due to lifestyle, behavior, occupation or known exposure events, or belonged to groups at risk for infection with HIV. Samples were from subjects in California, Georgia and Florida. Seventy-four (7.4%) of the 1004 samples were reactive with the VITROS HIV Combo test.

The VITROS HIV Combo test results among subjects at high risk for infection with HIV are presented in the following table:

Performance of the VITROS HIV Combo Test in U.S. Populations 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	Negative	IR	Reactive	
Adult	1004	932	72	72	930	74	74	71

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

Seventy-one (7.1%) of the 1004 samples from U.S. adult subjects at high risk for infection with HIV tested reactive with the VITROS HIV Combo test and positive by supplemental testing. All 71 of those samples testing supplemental positive were reactive with the FDA-approved HIV Ag/Ab Combo test.

The performance of the VITROS HIV Combo test compared with HIV status in the U.S. adult population at high risk for infection with HIV is summarized in the following table:

Agreement of the VITROS HIV Combo Test with HIV Status in U.S. Adult Populations at High Risk for Infection with HIV

VITROS HIV Combo Result	HIV Status		Total
	Positive	Negative	
Reactive	71	3	74
Negative	0	930	930
Total	71	933	1004

The sensitivity of the VITROS HIV Combo test was calculated as the percentage of the HIV positive samples that tested reactive with the test. 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 sensitivity of the VITROS HIV Combo test in the U.S. adult populations at high risk for infection with HIV was 100.00% in this study (71/71 with a 95% confidence interval (CI) of 94.94% to 100.00%). The specificity of the VITROS HIV Combo test in the U.S. adult populations at high risk for infection with HIV was 99.68% in this study (930/933 with a 95% confidence interval (CI) of 99.06% to 99.93%).

Among the 1004 U.S. adult subjects at high risk for infection with HIV participating in the clinical study, 559 (55.7%) reported no current signs or symptoms of infection with HIV. These individuals were from California, Georgia and Florida. These asymptomatic individuals were African American (70.5%) and Caucasian (27.5%), with the remaining 2.0% represented by other races. The group included 406 (72.6%) males and 153 (27.4%) females who ranged in age from 21 to

74. All were at risk due to lifestyle, behavior, occupation or known exposure events, or belonged to groups at risk for infection with HIV. The VITROS HIV Combo test results were reactive in 9.3% (52/559) of the individuals in the U.S. adult population at high risk for infection with HIV. The VITROS HIV Combo test reactive and negative results among the U.S. adult subjects at high risk for infection with HIV without signs or symptoms of infection with HIV by age and gender are shown in the following table:

Seroreactivity for the VITROS HIV Combo Test in U.S. Adult Subjects at High Risk for Infection with HIV without Signs or Symptoms of Infection with HIV

Age Range (years)	Gender	VITROS HIV Combo Results				Total
		Reactive		Negative		
		N	Percent	N	Percent	
21 - 29	Male	10	16.4	51	83.6	61
	Female	0	0.0	37	100.0	37
30 - 39	Male	10	12.2	72	87.8	82
	Female	5	10.6	42	89.4	47
40 - 49	Male	12	12.4	85	87.6	97
	Female	0	0.0	29	100.0	29
50 - 59	Male	9	7.4	113	92.6	122
	Female	2	6.3	30	93.8	32
60 - 69	Male	3	7.3	38	92.7	41
	Female	1	12.5	7	87.5	8
70 - 79	Male	0	0.0	3	100.0	3
	Female	0	0.0	0	0.0	0
Total		52	9.3	507	90.7	559

Among the 1004 U.S. adult subjects at high risk for infection with HIV participating in the clinical study, 445 (44.3%) reported signs or symptoms of infection with HIV. These individuals were from California, Georgia and Florida.

Reactivity in Populations at High Risk for Infection with HIV-2

The performance of the VITROS HIV Combo test was evaluated among individuals at high risk for infection with HIV-2 due to residence in an HIV-2 endemic area. The 522 subjects from this group were prospectively enrolled in West Africa.

Testing results are presented in the following table:

Performance of the VITROS HIV Combo Test in Individuals at High Risk for Infection with HIV-2

Population Description	Number Tested	FDA-Approved HIV Ag/Ab Combo Test			VITROS HIV Combo Test			Supplemental Positive
		NR	IR	R	Negative	IR	Reactive	
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 supplemental test positive. The FDA-

approved HIV Ag/Ab Combo test was reactive in 15 samples; 14 of these were supplemental test positive. 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 women.

The performance of the VITROS HIV Combo test compared with HIV status in the population at high risk for infection with HIV-2 is summarized in the following table:

Agreement of the VITROS HIV Combo Test with HIV Status in a Population at High Risk for Infection with HIV-2

VITROS HIV Combo Result	HIV Status		Total
	Positive	Negative	
Reactive	14	6	20
Negative	0	502	502
Total	14	508	522

The sensitivity of the VITROS HIV Combo test was calculated as the percentage of the HIV positive samples that tested reactive with the test. 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 sensitivity of the VITROS HIV Combo test in the population at high risk for infection with HIV-2 was 100.00% in this study (14/14 with a 95% confidence interval (CI) of 76.84% to 100.00%). The specificity of the VITROS HIV Combo test in the population at high risk for infection with HIV-2 was 98.82% in this study (502/508 with a 95% confidence interval (CI) of 97.45% to 99.57%).

The sensitivity of the VITROS HIV Combo test in adult populations at high risk, low risk and positive for infection with HIV is summarized in the following table:

Sensitivity in Adult Study Populations*

Population (Origin)	FDA-Approved HIV Ag/Ab Combo Test		VITROS HIV Combo Test	
	Sensitivity (%)	95% Exact Confidence Intervals	Sensitivity (%)	95% Exact Confidence Intervals
High Risk (U.S.)	100.00% (71/71)	94.94% - 100.00%	100.00% (71/71)	94.94% - 100.00%
High Risk (West Africa)	100.00% (14/14)	76.84% - 100.00%	100.00% (14/14)	76.84% - 100.00%
HIV Positive (U.S.)	100.00% (973/973)	99.62% - 100.00%	100.00% (973/973)	99.62% - 100.00%
HIV Positive (International)**	100.00% (459/459)	99.20% - 100.00%	100.00% (459/459)	99.20% - 100.00%
HIV-2 Positive (West Africa)	100.00% (229/229)	98.40% - 100.00%	100.00% (229/229)	98.40% - 100.00%
Low Risk (U.S.)	100.00% (44/44)	91.96% - 100.00%	100.00% (44/44)	91.96% - 100.00%
HIV-1 Antigen Positive***	94.23% (49/52)	84.05% - 98.79%	96.15% (50/52)	86.79% - 99.53%

* Data from pregnant women and pediatric subjects are not included in the table.

** In addition, 40/41 (97.56%) samples from International subjects positive for infection with HIV with sufficient volume only for VITROS HIV Combo testing were reactive with the test. 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.

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

The specificity of the VITROS HIV Combo test in adult populations at high risk and low risk for infection with HIV is summarized in the following table:

Specificity in Adult Study Populations*

Population	FDA-Approved HIV Ag/Ab Combo Test		VITROS HIV Combo Test	
	Specificity (%)	95% Exact Confidence Intervals	Specificity (%)	95% Exact Confidence Intervals
High Risk (U.S.)	99.89% (932/933)	99.40% - 100.00%	99.68% (930/933)	99.06% - 99.93%
High Risk (West Africa)	99.80% (507/508)	98.91% - 100.00%	98.82% (502/508)	97.45% - 99.57%
Low Risk (U.S.)	99.75% (5944/5959)	99.59% - 99.86%	99.58% (5934/5959)	99.38% - 99.73%

* Data from pregnant women and pediatric subjects are not included in the table.

Pregnant Women Populations

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

Specificity in Pregnant Women at Low Risk for Infection with HIV

Of the 262 pregnant women from California, Georgia and Florida 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.

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

Performance of the VITROS HIV Combo Test in Pregnant Women at Low 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	Negative	IR	Reactive	
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 performance of the VITROS HIV Combo test compared with HIV status in pregnant women at low risk for infection with HIV is summarized in the following table:

Agreement of the VITROS HIV Combo Test with HIV Status in Pregnant Women at Low Risk for Infection with HIV

VITROS HIV Combo Result	HIV Status		Total
	Positive	Negative	
Reactive	0	0	0
Negative	0	322	322
Total	0	322	322

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 was 100.00% in this study (322/322 with a 95% confidence interval (CI) of 98.86% to 100.00%).

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 an 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 VITROS HIV Combo test results among the samples from
U.S. pregnant women infected with HIV-1 are presented in the following table:

Performance of the VITROS HIV Combo Test in U.S. Pregnant Women Infected with HIV-1

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

* All samples were positive for anti-HIV-1

The sensitivity of the VITROS HIV Combo test in samples from U.S. pregnant women infected with HIV-1 was 100.00% (49/49; 95% CI = 92.75% to 100.00%).

Reactivity in 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 distribution of VITROS HIV Combo test results among pregnant women at high risk for infection with HIV is presented in the following table:

Seroreactivity for the VITROS HIV Combo Test in Pregnant Women at High Risk for Infection with HIV

Trimester	VITROS HIV Combo Results				Total
	Reactive		Negative		
	N	Percent	N	Percent	
First	1	1.1	90	98.9	91
Second	1	1.2	85	98.8	86
Third	0	0.0	86	100.0	86
Total	2	0.8	261	99.2	263

The VITROS HIV Combo test results in pregnant women at high risk for infection with HIV are presented in the following table:

Performance of the VITROS HIV Combo Test in U.S. Pregnant Women 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	Negative	IR	Reactive	
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 samples was reactive with the VITROS HIV Combo test and positive by supplemental testing. The one sample testing supplemental positive was reactive with the FDA-approved HIV Ag/Ab Combo test.

The performance of the VITROS HIV Combo test compared with HIV status in pregnant women at high risk for infection with HIV is summarized in the following table:

Agreement of the VITROS HIV Combo Test with HIV Status in Pregnant Women at High Risk for Infection with HIV

VITROS HIV Combo Result	HIV Status		Total
	Positive	Negative	
Reactive	1	1	2
Negative	0	261	261

The sensitivity of the VITROS HIV Combo test was calculated as the percentage of the HIV positive samples that tested reactive with the test. 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 sensitivity of the VITROS HIV Combo test in pregnant women at high risk for infection with HIV was 100.00% in this study (1/1, 95% confidence interval (CI) is not meaningful with sample size < 5). The specificity of the VITROS HIV Combo test in pregnant women at high risk for infection with HIV was 99.62% in this study (261/262 with a 95% confidence interval (CI) of 97.89% to 99.99%).

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

The sensitivity of the VITROS HIV Combo test in pregnant women at high risk and positive for infection with HIV is summarized in the following table:

Sensitivity in Pregnant Women Populations

Population	FDA-Approved HIV Ag/Ab Combo Test		VITROS HIV Combo Test	
	Sensitivity (%)	95% Exact Confidence Intervals	Sensitivity (%)	95% Exact Confidence Intervals
High Risk (U.S.)	100.00% (1/1)	N/A*	100.00% (1/1)	N/A*
HIV Positive (U.S.)	100.00% (49/49)	92.75% - 100.00%	100.00% (49/49)	92.75% - 100.00%
Total	100.00% (50/50)	92.89% - 100.00%	100.00% (50/50)	92.89% - 100.00%

* Sample size <5; Confidence interval is not meaningful.

The specificity of the VITROS HIV Combo test in pregnant women at high risk and low risk for infection with HIV is summarized in the following table:

Specificity in Pregnant Women Populations

Population	FDA-Approved HIV Ag/Ab Combo Test		VITROS HIV Combo Test	
	Specificity (%)	95% Exact Confidence Intervals	Specificity (%)	95% Exact Confidence Intervals
High Risk (U.S.)	100.00% (262/262)	98.60% - 100.00%	99.62% (261/262)	97.89% - 99.99%
Low Risk (U.S.)	99.62% (261/262)	97.89% - 99.99%	100.00% (262/262)	98.60% - 100.00%
Labor and Delivery (U.S.)	100.00% (60/60)	94.04% - 100.00%	100.00% (60/60)	94.04% - 100.00%
Total	99.83% (583/584)	99.05% - 100.00%	99.83% (583/584)	99.05% - 100.00%

Pediatric Populations

Four hundred fifteen (415) samples from pediatric subjects were tested with the VITROS HIV Combo test and an FDA- approved HIV Ag/Ab Combo test (with supplemental testing as required). An additional 11 samples only had sufficient volume for VITROS HIV Combo testing. The samples were from individuals at low risk for infection with HIV (N=110),

individuals at high risk for infection with HIV (N=251) and individuals positive for infection with HIV (N=54, plus samples with volume only for VITROS HIV Combo testing, N=11).

Specificity in Pediatric Subjects at Low Risk for Infection with HIV

Samples from 110 pediatric subjects in groups at low risk for infection with HIV were from Florida. Of the 110 pediatric subjects at low risk for infection with HIV, 59.1% were male and 40.9% were female and they ranged in age from 2-20 years. One (0.9%) of the 110 samples was reactive with the VITROS HIV Combo test. The distribution of VITROS HIV Combo test results among the pediatric subjects at low risk for infection with HIV by age and gender is presented in the following table:

Seroreactivity for the VITROS HIV Combo Test in Pediatric Subjects at Low Risk for Infection with HIV

Age Range (years)	Gender	VITROS HIV Combo Results				Total
		Reactive		Negative		
		N	Percent	N	Percent	
2 - 5	Male	0	0.0	9	100.0	9
	Female	0	0.0	10	100.0	10
6 - 10	Male	0	0.0	13	100.0	13
	Female	0	0.0	6	100.0	6
11 - 15	Male	0	0.0	18	100.0	18
	Female	0	0.0	15	100.0	15
16 - 20	Male	1	4.0	24	96.0	25
	Female	0	0.0	14	100.0	14
Total		1	0.9	109	99.1	110

The results obtained from the 110 samples from pediatric subjects at low risk for infection with HIV are summarized in the following table:

Performance of the VITROS HIV Combo Test in Pediatric Subjects at Low 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	Negative	IR	Reactive	
Pediatric	110	110	0	0	109	1	1	0

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

The performance of the VITROS HIV Combo test compared with HIV status in pediatric subjects at low risk for infection with HIV is summarized in the following table:

Agreement of the VITROS HIV Combo Test with HIV Status in Pediatric Subjects at Low Risk for Infection with HIV

VITROS HIV Combo Result	HIV Status		Total
	Positive	Negative	
Reactive	0	1	1
Negative	0	109	109
Total	0	110	110

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 pediatric subjects at low risk for infection with HIV was 99.09% in this study (109/110 with a 95% confidence interval (CI) of 95.04% to 99.98%).

Sensitivity in Samples from 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 an FDA-approved HIV Ag/Ab Combo test. An additional 11 samples only had sufficient volume for VITROS HIV Combo testing. The pediatric subjects ranged in age from 2 to 20 years. The VITROS HIV Combo test results among these samples are presented in the following table:

Performance of the VITROS HIV Combo Test in Samples from Pediatric Subjects Infected with HIV

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

* All samples were positive for anti-HIV-1

** 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 were reactive with the VITROS HIV Combo test. The sensitivity of the VITROS HIV Combo test for U.S. pediatric samples enrolled as infected with HIV-1 was 98.15% (53/54; 95% CI = 90.11% to 99.95%).

An additional 11 U.S. pediatric samples from subjects infected with HIV-1 had sufficient volume only for VITROS HIV Combo testing and 11/11 (100.00%) were reactive.

Reactivity in Pediatric Subjects at High Risk for Infection with HIV

Of the 251 pediatric subjects at high risk for infection with HIV, 46.2% were male and 53.8% were female and they ranged in age from 2-20 years. These subjects were enrolled in Georgia and Puerto Rico. Seven (2.8%) of the 251 samples were reactive with the VITROS HIV Combo test. The distribution of VITROS HIV Combo test results among the pediatric subjects at high risk for infection with HIV by age and gender is presented in the following table:

Seroreactivity for the VITROS HIV Combo Test in the Pediatric Subjects at High Risk for Infection with HIV

Age Range (years)	Gender	VITROS HIV Combo Results				Total
		Reactive		Negative		
		N	Percent	N	Percent	
2 - 5	Male	1	6.3	15	93.8	16
	Female	0	0.0	14	100.0	14
6 - 10	Male	0	0.0	27	100.0	27
	Female	1	3.8	25	96.2	26
11 - 15	Male	0	0.0	27	100.0	27
	Female	0	0.0	30	100.0	30
16 - 20	Male	2	4.3	44	95.7	46
	Female	3	4.6	62	95.4	65
Total		7	2.8	244	97.2	251

The VITROS HIV Combo test results in pediatric subjects at high risk for infection with HIV are presented in the following table:

Performance of the VITROS HIV Combo Test in U.S. Pediatric Subjects 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	Negative	IR	Reactive	
Pediatric	251	248	3	3	244	8	7	3

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

Three (1.2%) of the 251 pediatric samples from subjects at high risk for infection with HIV tested reactive with the VITROS HIV Combo test and positive by supplemental testing. All three of those samples testing supplemental positive were reactive with the FDA-approved HIV Ag/Ab Combo test.

The performance of the VITROS HIV Combo test compared with HIV status in pediatric subjects at high risk for infection with HIV is summarized in the following table:

Agreement of the VITROS HIV Combo Test with HIV Status in Pediatric Subjects at High Risk for Infection with HIV

VITROS HIV Combo Result	HIV Status		Total
	Positive	Negative	
Reactive	3	4	7
Negative	0	244	244
Total	3	248	251

The sensitivity of the VITROS HIV Combo test was calculated as the percentage of the HIV positive samples that tested reactive with the test. 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 sensitivity of the VITROS HIV Combo test in pediatric subjects at high risk for infection with HIV was 100.00% in this study (3/3, 95% confidence interval (CI) is not meaningful with sample size < 5). The specificity of the VITROS HIV Combo test in pediatric subjects at high risk for infection with HIV was 98.39% in this study (244/248 with a 95% confidence interval (CI) of 95.92% to 99.56%).

The sensitivity of the VITROS HIV Combo test in pediatric subjects at high risk and positive for infection with HIV is summarized in the following table:

Sensitivity in Pediatric Populations

Population	FDA-Approved HIV Ag/Ab Combo Test		VITROS HIV Combo Test	
	Sensitivity (%)	95% Exact Confidence Intervals	Sensitivity (%)	95% Exact Confidence Intervals
High Risk (U.S.)	100.00% (3/3)	N/A*	100.00% (3/3)	N/A*
HIV Positive (U.S.)	98.15% (53/54)	90.11% - 99.95%	98.15% (53** /54)	90.11% - 99.95%
Total	98.25% (56/57)	90.61% - 99.96%	98.25% (56/57)	90.61% - 99.96%

* Sample size <5; Confidence interval is not meaningful.

** Sample volume was insufficient for supplemental testing. The sample was assigned an HIV status of positive based on the certificate of analysis.

The specificity of the VITROS HIV Combo test in pediatric subjects at high risk and low risk for infection with HIV is summarized in the following table:

Specificity in Pediatric Populations

Population	FDA-Approved HIV Ag/Ab Combo Test		VITROS HIV Combo Test	
	Specificity (%)	95% Exact Confidence Intervals	Specificity (%)	95% Exact Confidence Intervals
Low Risk (U.S.)	100.00% (110/110)	96.70% - 100.00%	99.09% (109/110)	95.04% - 99.98%
High Risk (U.S.)	100.00% (248/248)	98.52% - 100.00%	98.39% (244/248)	95.92% - 99.56%
Total	100.00% (358/358)	98.97% - 100.00%	98.60% (353/358)	96.77% - 99.54%

Seroconversion Panels

Thirty-two commercially available seroconversion panels were tested on both the VITROS HIV Combo test and a commercially available FDA-approved HIV Ag/Ab Combo test. Results for the thirty-two 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 and the difference in days to first reactive between the two tests. Data are presented for both tests for each of the seroconversion panels.

Panel ID	Number of Panel Members Tested	Number of Reactive Panel Members		FDA-Approved HIV Ag/Ab Combo Test		VITROS HIV Combo Test		Difference in Days to First Reactive Result
		FDA-Approved HIV Ag/Ab Combo Test	VITROS HIV Combo Test	Day of Last Non-reactive Result*	Day of First Reactive Result**	Day of Last Negative Result*	Day of First Reactive Result**	FDA-Approved HIV Ag/Ab Combo 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

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

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

** Post bleed day of first reactive result.

Detection of HIV-1 Antigen Genotypes

Three HIV-1 group M subtype specimens and 49 viral isolates were tested with the VITROS HIV Combo test. The reactivity by HIV-1 antigen subtype and country of origin on the VITROS 3600 Immunodiagnostic System and an FDA-approved HIV Ag/Ab Combo test is presented in the table below.

Source (# of Specimens)	Subtype	Number of Specimens Tested	VITROS HIV Combo Test	FDA-Approved HIV Ag/Ab Combo Test
			Number of Reactive Interpretations (%)	Number of Reactive Interpretations (%)
Group M Specimens (3)				
Uganda	A	1	1 (100)	1 (100)
Uganda	D	1	1 (100)	1 (100)
Romania	F	1	1 (100)	1 (100)
Viral Isolates * (49)				
Ghana (1), Kyrgyzstan(1), Uganda (2)	A	4	4 (100)	4 (100)
Brazil (1), Thailand (1), USA (6)	B	8	8 (100)	6 (75.0)
Djibouti (1), Ethiopia (1), Senegal (1), Somalia (1), Uganda (1), Zambia (1), Unknown (1)	C	7	5 (71.4)	4 (57.1)
Senegal (1), Uganda (2)	D	3	3 (100)	3 (100)
Brazil (3), Romania (2)	F	5	5 (100)	4 (80.0)
Kenya (1), Democratic Republic of the Congo (1)	G	2	2 (100)	2 (100)
Democratic Republic of the Congo (1)	H	1	1 (100)	1 (100)
Indonesia (2), Thailand (8)	CRF01_AE	10	10 (100)	10 (100)
Djibouti (2), Liberia (1)	CRF02_AG	3	3 (100)	3 (100)
Cameroon (2), USA (1), Spain (1)	Group O	4	4 (100)	4 (100)
Unknown	IIIB	1	1 (100)	0 (0.0)
Unknown	HIV-2	1	1 (100)	1 (100)
Total		52	50 (96.2)	45 (86.5)

* Isolates tested after dilution to 200,000 RNA copies/mL in Defibrinated, Delipidized Plasma

Detection of HIV-1 p24 Antigen Standards

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 two 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 0.48 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 (90/636) and 12.8 pg/mL (range 12.6 to 13.1 pg/mL) for the AFSSAPS HIV-1 Antigen standard.

Potentially Cross-Reacting Subgroups

The VITROS HIV Combo test was evaluated for potential cross-reactivity in HIV negative samples from medical conditions unrelated to HIV infection. The results are summarized in the table below.

Sample Category	Number Tested	Number Negative	Number Reactive	Number Confirmed Positive*
HCV Antigen	6	6	0	N/A
HCV Antibody	10	10	0	N/A
HBsAg	6	6	0	N/A
HBc Antibody	10	10	0	N/A
HTLV I Antigen	6	6	0	N/A
HTLV II Antigen	6	6	0	N/A
HTLV I Antibody	6	6	0	N/A
HTLV II Antibody	6	6	0	N/A
EBV Antigen	6	6	0	N/A
EBV Antibody	10	10	0	N/A
HSV I Antigen	6	6	0	N/A

Sample Category	Number Tested	Number Negative	Number Reactive	Number Confirmed Positive*
HSV II Antigen	6	6	0	N/A
HSV Antibody	12	12	0	N/A
Chlamydia	10	10	0	N/A
Gonorrhea	10	10	0	N/A
Syphilis	10	10	0	N/A
Multiparous Female	10	10	0	N/A
Pregnant Women (1st Trimester)	8	8	0	N/A
Pregnant Women (2nd Trimester)	8	8	0	N/A
Pregnant Women (3rd Trimester)	8	8	0	N/A
Pre Flu Vaccine	6	5	1	1
Post Flu Vaccine	6	5	1	1
Influenza A Antigen	6	6	0	N/A
Influenza B Antigen	6	6	0	N/A
Rheumatoid Factor (RF)	11	11	0	N/A
Human Anti-Mouse Antibody (HAMA)	10	10	0	N/A
Autoimmune Disease	10	10	0	N/A
Anti-Nuclear Antibodies (ANA)	10	10	0	N/A
Hemophilia	20	11	9	9
Dialysis	10	10	0	N/A
Yeast Reactive: Candida	10	10	0	N/A
Super-oxide Dismutase (SOD)	1	1	0	N/A
Cytomegaloviru s Antigen	6	6	0	N/A
Cytomegaloviru s Antibody	10	10	0	N/A
Toxoplasma infection	10	10	0	N/A
HAV Antigen	6	6	0	N/A
HAV Antibody	10	10	0	N/A
Rubella infection	10	10	0	N/A
Elevated IgG	22	21	1	1
Elevated IgM	10	10	0	N/A
Non-Viral Liver Disease	10	10	0	N/A
Pediatric (2-5 yrs.)	4	4	0	N/A
Pediatric (6-10 yrs.)	6	6	0	N/A
Pediatric (11-16 yrs.)	6	6	0	N/A
Pediatric (17-21 yrs.)	4	4	0	N/A
Elevated Cholesterol	21	20	1	1
Elevated Total Protein	14	10	4	4
Elevated Triglycerides	17	14	3**	2

* Confirmed as HIV positive using VITROS Anti-HIV 1+2, Bio-Rad MultiSpot and Aptima Qualitative HIV-1 RNA

** One sample was negative on Bio-Rad MultiSpot and not detected on Aptima Qualitative HIV-1 RNA.

Potentially Cross-Reacting Sub-Groups - Microbiological Studies

The potential for bacterial contamination to affect the performance of the VITROS HIV Combo test was evaluated further by testing samples spiked with Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. 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 that were tested none of the HIV negative samples were found to be false reactive and none of the HIV spiked samples were observed to be false negative in the VITROS HIV Combo test.

Specificity

Substances that do not Interfere

The VITROS HIV Combo test was evaluated for interference consistent with CLSI document EP7. [7] Of the compounds tested, none was found to interfere with the clinical interpretation of the test in negative and weakly reactive samples at the concentrations indicated.

Test Substance	Maximum Level tested	
Bilirubin (conjugated)	30 mg/dL	0.386 mmol/L
Bilirubin (unconjugated)	30 mg/dL	0.513 mmol/L
Biotin	3000 ng/mL	12300 nmol/L
Hemoglobin	500 mg/dL	0.078 mmol/L
Cholesterol	300 mg/dL	77.7 mmol/L
HAMA	263 ng/mL	N/A
IgG	2380 mg/dL	23.80 g/L
RF	3020 IU/mL	N/A
Total Protein	10.9 g/dL	109 g/L
Triglycerides	1250 mg/dL	14.13 mmol/L
Intralipid	850 mg/dL	N/A

N/A = alternate units not provided

Precision

Precision was evaluated consistent with CLSI document EP05. [11] Two replicates each of 14 negative or diluted reactive patient sample pools and 5 control samples were tested on 2 separate occasions per day on at least 20 different days. The experiment was performed using 3 reagent lots on 3 different VITROS 3600 Immunodiagnostic Systems.

Panel Member	Mean VITROS HIV Combo Test Results (S/C)	Between Instruments [a]		Between Lot [b]		Between Calibration [c]		Between Day [d]		Between Run [e]		Within Run / Residual [f]		Total [g]		No. Observ.
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	
Negative[i]	0.09	0.012	N/A[h]	0.024	N/A[h]	0.004	N/A[h]	0.009	N/A[h]	0.000	N/A[h]	0.024	N/A[h]	0.038	N/A[h]	524
Negative[j]	0.10	0.010	N/A[h]	0.025	N/A[h]	0.004	N/A[h]	0.009	N/A[h]	0.000	N/A[h]	0.017	N/A[h]	0.033	N/A[h]	524
Negative Control[k]	0.09	0.016	N/A[h]	0.017	N/A[h]	0.004	N/A[h]	0.009	N/A[h]	0.000	N/A[h]	0.026	N/A[h]	0.036	N/A[h]	524
Anti-HIV-1	0.67	0.000	0.0	0.086	13.0	0.021	3.1	0.015	2.3	0.004	0.5	0.052	7.8	0.104	15.7	528
Anti-HIV-1	1.14	0.000	0.0	0.100	8.7	0.036	3.1	0.000	0.0	0.021	1.9	0.080	7.1	0.135	11.8	528
Anti-HIV-1	2.43	0.000	0.0	0.200	8.2	0.059	2.4	0.000	0.0	0.025	1.0	0.127	5.2	0.245	10.1	528
Anti-HIV-1 Control	1.99	0.000	0.0	0.059	3.0	0.046	2.3	0.035	1.7	0.000	0.0	0.120	6.0	0.145	7.3	520
Anti-HIV-2	0.83	0.018	2.1	0.135	16.2	0.026	3.1	0.026	3.1	0.000	0.0	0.073	8.7	0.158	19.0	524
Anti-HIV-2	1.18	0.000	0.0	0.173	14.7	0.041	3.5	0.032	2.7	0.000	0.0	0.085	7.2	0.200	17.0	528
Anti-HIV-2	2.66	0.000	0.0	0.241	9.1	0.054	2.0	0.027	1.0	0.013	0.5	0.152	5.7	0.291	11.0	528
Anti-HIV-2 Control	4.42	0.000	0.0	0.325	7.3	0.090	2.0	0.000	0.0	0.021	0.5	0.203	4.6	0.394	8.9	528
Anti-HIV-1 Group O	1.03	0.000	0.0	0.191	18.5	0.029	2.8	0.013	1.3	0.006	0.6	0.082	7.9	0.211	20.4	528
Anti-HIV-1 Group O	1.28	0.000	0.0	0.204	16.0	0.033	2.6	0.019	1.5	0.001	0.1	0.093	7.2	0.227	17.8	528
Anti-HIV-1 Group O	2.72	0.000	0.0	0.322	11.8	0.062	2.3	0.000	0.0	0.035	1.3	0.165	6.1	0.369	13.5	528
Anti-HIV-1 Group O Control	3.24	0.075	2.3	0.102	3.1	0.066	2.0	0.000	0.0	0.025	0.8	0.145	4.5	0.205	6.3	528
HIV p24 Ag	0.81	0.006	0.7	0.014	1.8	0.023	2.8	0.018	2.2	0.009	1.2	0.055	6.8	0.064	8.0	528
HIV p24 Ag	1.44	0.000	0.0	0.074	5.1	0.034	2.3	0.020	1.4	0.018	1.2	0.076	5.3	0.115	8.0	528
HIV p24 Ag[l]	3.32	0.000	0.0	0.261	7.9	0.065	2.0	0.010	0.3	0.013	0.4	0.147	4.4	0.307	9.3	524
HIV p24 Ag Control	1.88	0.000	0.0	0.118	6.3	0.046	2.4	0.039	2.1	0.000	0.0	0.099	5.3	0.166	8.8	528

[a] Between-instrument: Variability of the test performance from instrument to instrument.

[b] Between-lot: Variability of the test performance from lot to lot.

[c] Between-calibration: Variability of the test performance from calibration to calibration within instrument.

[d] Between-day: Variability of the test performance from day to day.

[e] Between-run: Variability of the test performance from run to run.

[f] Within-run / Residual Variability.

[g] Total: Variability of the test incorporating factors of Instrument, lot, calibration, day and run.

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

[i] One replicate had a S/C ratio of 1.38 which was a statistical outlier. The entire test day for the panel member was excluded from analysis. When this replicate was included, the mean S/C ratio was 0.09, %CV are not meaningful when S/C approaches zero.

[j] One replicate had a S/C ratio of 1.17 which was a statistical outlier. The entire test day for the panel member was excluded from analysis. When this replicate was included, the mean S/C ratio was 0.10, %CV are not meaningful when S/C approaches zero.

[k] One replicate had a S/C ratio of 0.45 which was a statistical outlier. The entire test day for the panel member was excluded from analysis. When this replicate was included, the mean S/C ratio was 0.09, %CV are not meaningful when S/C approaches zero.

[l] One replicate had a S/C ratio of 20.0 which was a statistical outlier. The entire test day for the panel member was excluded from analysis. When this replicate was included, the mean S/C ratio was 3.35, the Between Instruments CV (%) was 0.0, the Between Lot CV (%) was 8.9, the Between Calibration CV (%) was 0.0, the Between Day CV (%) was 0.0, the Between Run CV (%) was 4.2, the within- Run CV (%) was 22.0, and the Total CV (%) was 24.1.

Reproducibility

Precision was further evaluated incorporating between run, between day, between site and between lot variations. The 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. The data in the table shown were rounded following all calculations.

Panel Member	Grand Mean (S/C)	Within Run		Between - Run/ Operator		Between - Day		Within-Laboratory (Total)[a]		Between Site[b]		Between Lot[c]		Overall[d]		No. Obs.
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	
Negative	0.12	0.050	N/A[e]	0.000	N/A[e]	0.026	N/A[e]	0.057	N/A[e]	0.019	N/A[e]	0.025	N/A[e]	0.066	N/A[e]	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[f]	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[g]	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[e]	0.004	N/A[e]	0.026	N/A[e]	0.040	N/A[e]	0.017	N/A[e]	0.024	N/A[e]	0.051	N/A[e]	324

[a] Within-Laboratory (Total) variability contains the Within-Run, Between-Run/Operator, and Between-Day variance components.

[b] Between site: Variability of the test performance from site to site.

[c] Between lot: Variability of the test performance from lot to lot, calculated using data across all sites.

[d] Total: Variability of the test incorporating factors of site, lot, operator and day.

[e] N/A = Not applicable. % CV are not meaningful when S/C approaches zero

[f] 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 (%) was 46.8, the Between Site CV (%) was 0.0, the Between Lot CV (%) was 8.8, and the Overall CV (%) was 48.1.

[g] 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.

Precision

VITROS 5600 Integrated System

Precision was evaluated consistent with CLSI document EP5. [11] Two replicates each of 14 frozen negative or diluted reactive patient sample pools and 5 control samples[a] were tested on 2 separate occasions per day on at least 20 different days. The experiment was performed using 1 reagent lot on each system. The data presented are a representation of the product performance.

System	Panel Member	Mean VITROS HIV Combo Test Results (S/C)	Within Run *		Within Calibration* *		Within-lab***		No. Observ.	No. Days
			SD	CV (%)	SD	CV (%)	SD	CV (%)		
3600	Negative[b]	0.09	0.037	N/A	0.040	N/A	0.039	N/A	84	21
	Negative	0.09	0.009	N/A	0.016	N/A	0.017	N/A	88	22
	Negative Control	0.08	0.007	N/A	0.014	N/A	0.015	N/A	88	22
	Anti-HIV-1	0.69	0.022	3.1	0.054	7.7	0.056	8.2	88	22
	Anti-HIV-1	1.20	0.033	2.7	0.081	6.6	0.085	7.2	88	22
	Anti-HIV-1	2.63	0.050	1.9	0.122	4.6	0.126	4.9	88	22
	Anti-HIV-1 Control	2.10	0.046	2.2	0.094	4.4	0.106	5.1	88	22
	Anti-HIV-2	0.80	0.044	5.4	0.081	10.0	0.081	10.3	88	22
	Anti-HIV-2	1.14	0.044	3.8	0.087	7.5	0.090	8.0	88	22
	Anti-HIV-2	2.65	0.059	2.2	0.121	4.5	0.132	5.1	88	22
	Anti-HIV-2 Control	4.30	0.063	1.4	0.166	3.8	0.195	4.6	88	22
	Anti-HIV-1 Group O	1.01	0.031	3.0	0.067	6.6	0.070	7.1	88	22
	Anti-HIV-1 Group O	1.25	0.046	3.6	0.084	6.6	0.087	7.1	88	22
	Anti-HIV-1 Group O	2.72	0.069	2.5	0.144	5.2	0.147	5.5	88	22
	Anti-HIV-1 Group O Control	3.20	0.071	2.2	0.120	3.7	0.137	4.3	88	22
	HIV p24 Ag	0.81	0.019	2.3	0.064	7.8	0.066	8.3	88	22
	HIV p24 Ag	1.50	0.031	2.0	0.088	5.8	0.091	6.2	88	22
	HIV p24 Ag	3.50	0.062	1.7	0.119	3.4	0.141	4.1	88	22
	HIV p24 Ag Control	1.96	0.034	1.7	0.112	5.6	0.115	6.0	88	22

System	Panel Member	Mean VITROS HIV Combo Test Results (S/C)	Within Run *		Within Calibration**		Within-lab***		No. Observ.	No. Days
			SD	CV (%)	SD	CV (%)	SD	CV (%)		
5600	Negative[c]	0.06	0.008	N/A	0.011	N/A	0.010	N/A	80	20
	Negative	0.08	0.014	N/A	0.014	N/A	0.015	N/A	84	21
	Negative Control[d]	0.07	0.011	N/A	0.015	N/A	0.016	N/A	80	20
	Anti-HIV-1[e]	0.66	0.050	7.9	0.073	11.6	0.067	9.9	80	20
	Anti-HIV-1	1.14	0.028	2.6	0.070	6.4	0.061	5.2	84	21
	Anti-HIV-1	2.51	0.045	1.9	0.132	5.5	0.108	4.1	84	21
	Anti-HIV-1 Control	2.01	0.160	8.3	0.188	9.7	0.217	10.4	84	21
	Anti-HIV-2[f]	0.75	0.029	4.0	0.066	9.2	0.062	7.9	80	20
	Anti-HIV-2	1.07	0.039	3.8	0.092	9.0	0.080	7.2	84	21
	Anti-HIV-2	2.52	0.049	2.0	0.140	5.8	0.134	5.1	84	21
	Anti-HIV-2 Control	4.11	0.058	1.5	0.186	4.7	0.190	4.4	84	21
	Anti-HIV-1 Group O	0.92	0.031	3.5	0.067	7.6	0.062	6.5	84	21
	Anti-HIV-1 Group O	1.15	0.043	3.9	0.084	7.6	0.072	6.1	84	21
	Anti-HIV-1 Group O[g]	2.56	0.060	2.4	0.139	5.7	0.130	4.9	80	20
	Anti-HIV-1 Group O Control	3.11	0.239	8.0	0.277	9.3	0.300	9.3	84	21
	HIV p24 Ag	0.81	0.024	3.1	0.073	9.5	0.071	8.5	84	21
	HIV p24 Ag	1.49	0.043	3.0	0.097	6.8	0.086	5.5	84	21
	HIV p24 Ag	3.51	0.044	1.3	0.165	4.9	0.181	5.0	84	21
	HIV p24 Ag Control	1.96	0.030	1.6	0.099	5.3	0.093	4.6	84	21

* Within-Run (repeatability): between duplicate precision averaged over all runs.

** Within calibration: Total precision with weighted components of within-run, between-run and between-day variation.

*** Within-lab: A measure of the effect of recalibration on total precision, calculated within reagent lot, using data from at least 4 calibrations.

**** Performance characteristics for the VITROS 5600 System are applicable to the VITROS XT 7600 System.

[a] The Controls used were Bio-Rad VIROTROL Controls

[b] One test day removed due to statistical outlier (1.46 S/C) [c] One test day removed due to statistical outlier (2.08 S/C) [d] One test day removed due to statistical outlier (12.1 S/C) [e] One test day removed due to statistical outlier (3.11 S/C) [f] One test day removed due to statistical outlier (6.19 S/C) [g] One test day removed due to statistical outlier (7.36 S/C)

N/A = Not Applicable

Percent Agreement Between VITROS 3600 Immunodiagnostic System and VITROS 5600 Integrated System

Percent agreement was evaluated in a method comparison study using 685 serum and plasma samples (502 negative, 130 anti-HIV-1 reactive, 40 anti-HIV-2 reactive, 6 anti-HIV-1 group O reactive and 7 HIV p24 antigen reactive). Results from samples analyzed on the VITROS 3600 Immunodiagnostic System were compared with those analyzed on the VITROS 5600 Integrated System at 3 different sites with a single reagent lot. Positive and negative percent confidence intervals were determined using a bootstrap method to account for identical samples used at each site.

Percent Agreement of Samples to VITROS 3600 Immunodiagnostic System

System	Positive Agreement (≥ 1.00 S/C)	95% Confidence Interval	Negative Agreement (< 1.00 S/C)	95% Confidence Interval
5600	95.7%	93.7-97.5%	97.1%	95.9-98.1%

**** Performance characteristics for the VITROS 5600 System are applicable to the VITROS XT 7600 System.

Patent Statements

HIV-1 and HIV-2 recombinant antigens used in the VITROS HIV Combo test are prepared under US license by Grifols Diagnostic Solutions Inc. under a shared manufacturing agreement.

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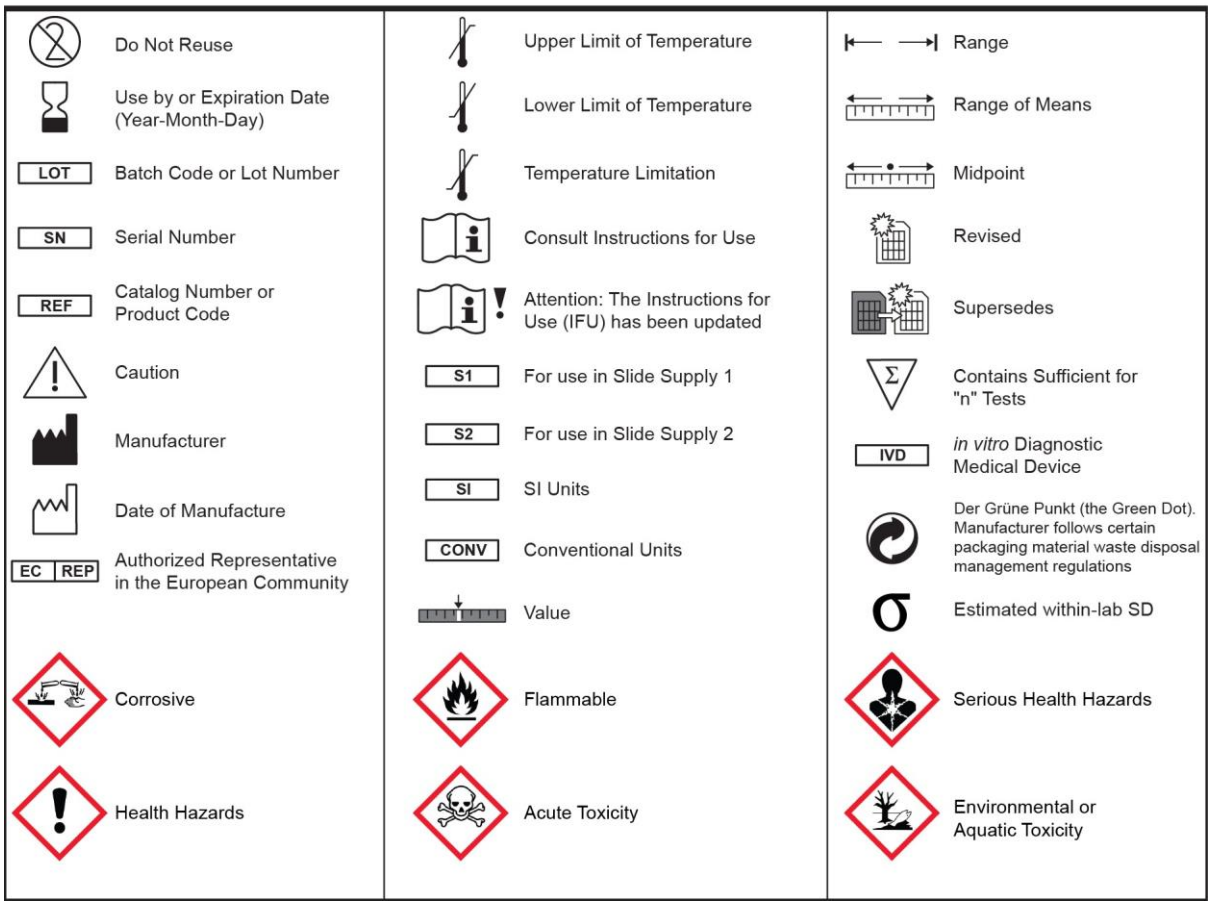
Glossary of Symbols

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



Revision History

Date of Revision	Version	Description of Technical Changes*
2018-11-08	Draft	Draft for PMA Supplement
2018–11–05	2.0	Added Information for the VITROS 5600 Integrated System
2018–01–05	1.0	Initial version of Instructions for Use.

* The change bars indicate the position of a technical amendment to the text with respect to the previous version of the document.

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

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