VITROS Immunodiagnostic Products

Not Intended for Use in Canada

INSTRUCTIONS FOR USE HIV c

VITROS Immunodiagnostic Products HIV Combo Reagent Pack	REF	684 2779
VITROS Immunodiagnostic Products HIV Combo Calibrator	REF	684 2780

Intended Use

For *in vitro* diagnostic use only.

VITROS Immunodiagnostic Products HIV Combo Reagent Pack

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, using the VITROS ECi/ECiQ/3600 Immunodiagnostic Systems and the VITROS 5600/XT 7600 Integrated Systems.

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 in persons at high and low risk for HIV infection and as a screening test for the detection of HIV-1 and/or HIV-2 in blood donors.

VITROS Immunodiagnostic Products HIV Combo Calibrator

For use in the calibration of the VITROS ECi/ECiQ/3600 Immunodiagnostic Systems and the VITROS 5600/XT 7600 Integrated Systems 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.

Summary and Explanation of the Test

Acquired Immunodeficiency Syndrome (AIDS) is caused by at least 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 (env 13), HIV-1 group O envelope (env 70-3) and HIV–2 envelope (env 31). 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 in early seroconversion before the onset of antibody response enabling earlier diagnosis of HIV infection.

Principles of the Procedure

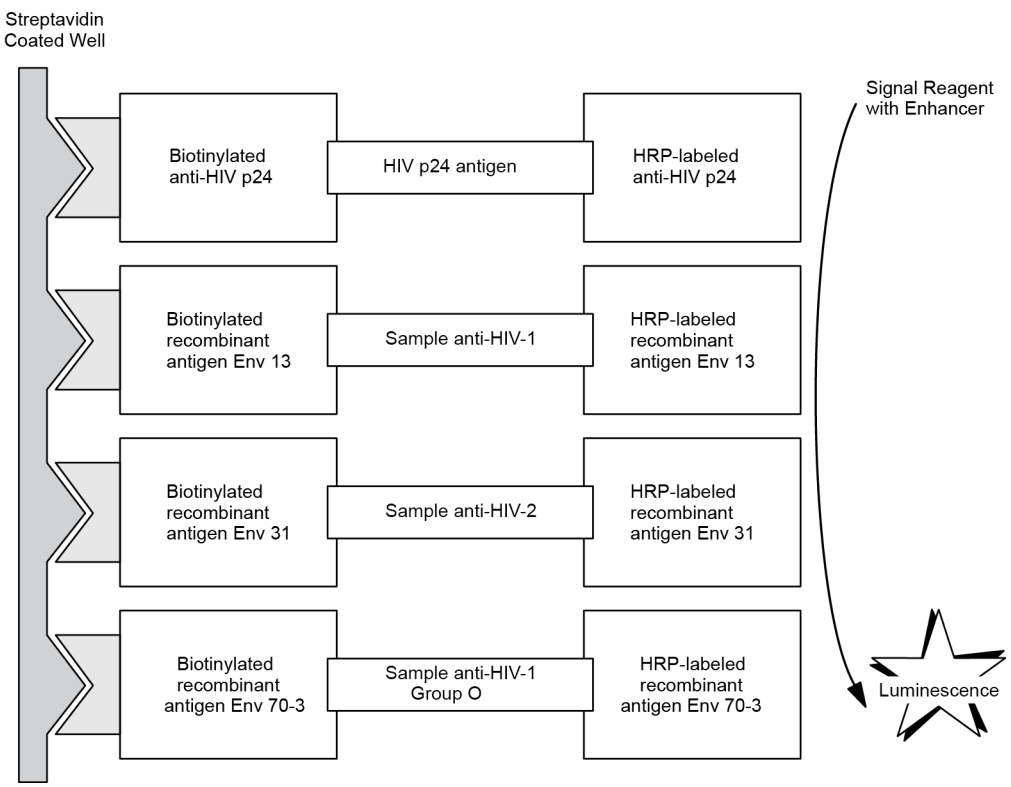
An immunometric technique is used; this involves a two stage reaction. In the first stage HIV antibody or antigen present in the sample binds with biotinylated HIV recombinant antigen or biotinylated antibody coated on streptavidin wells. Unbound sample is removed by washing. In the second stage horseradish peroxidase (HRP)-labeled recombinant HIV antigens and antibodies are added in the conjugate reagent. 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.

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

* Not all products and systems are available in all countries.

Reaction Scheme



Warnings and Precautions

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, 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 approved 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 high and low 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
- Lot calibration card
- Protocol card
- 8 calibrator bar code labels

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

- 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 Immunodiagnostic and VITROS Integrated Systems. Alternatively, transfer an aliquot of each 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
- Sodium citrate plasma
- Acid Citrate Dextrose (ACD)
- Citrate Phosphate Dextrose (CPD)
- Citrate Phosphate Dextrose Adenine (CPDA-1)

Note: The liquid citrate anticoagulants tested (sodium citrate plasma, ACD, CPD and CPDA-1) have no clinically significant effect on negative samples. However HIV reactive samples will show proportionally lower signal/cutoff values, due to dilution by the liquid anticoagulant. High negative results (0.70–0.99) obtained on samples collected with these anticoagulants should be interpreted accordingly. Supplemental tests may be required.

Specimens Not Recommended

Do not use turbid specimens. Turbidity in specimens may affect test results.

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, 6]
- 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, sodium heparin plasma, sodium citrate plasma, ACD, CPD and CPDA-1 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.

Testing Procedure

Materials Provided

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

Materials Required but Not Provided

- 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; reagent packs and calibrators are linked by lot number. Reagent packs from the same lot may use the same calibration.
- 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 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, 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

- Calibrate when the reagent pack and calibrator lot changes.
- Calibrate 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 the clinical sensitivity and specificity performance.

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 Immunodiagnostic and VITROS Integrated Systems.

Quality Control

Quality Control Material Selection

External controls may be used in accordance with local, government regulations or accrediting organizations, as applicable. Controls with 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 Immunodiagnostic and VITROS Integrated Systems

The performance of commercial control fluids 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. [7]

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

Quality Control Material Preparation and Storage

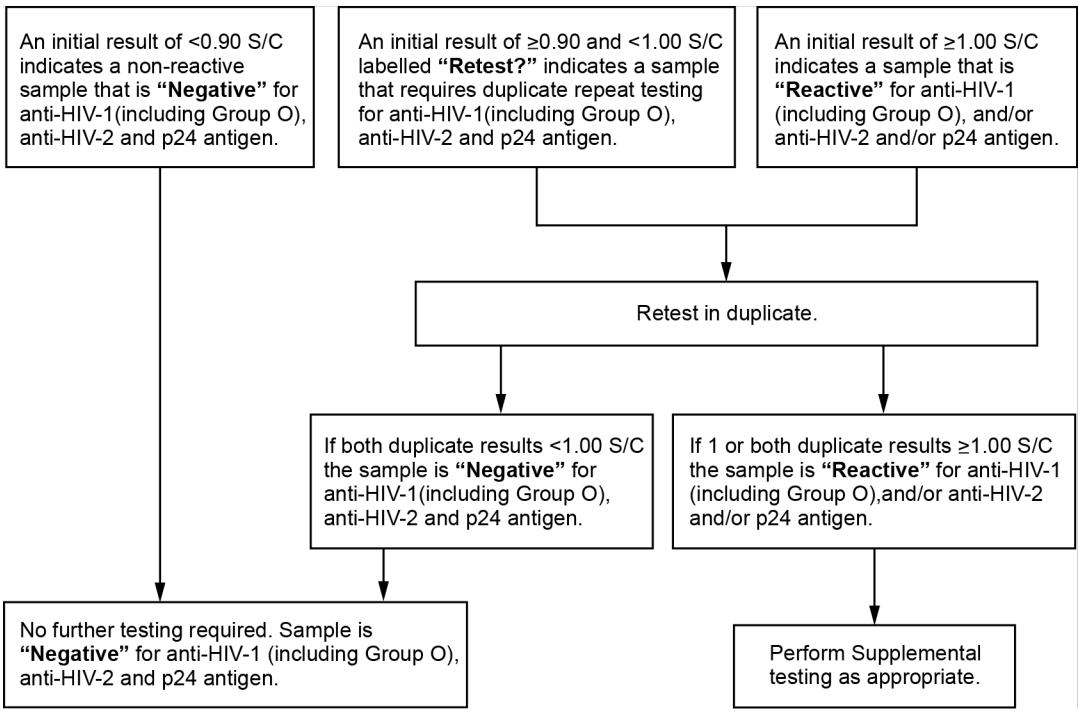
Refer to the manufacturer's product literature for preparation, storage, and stability information.

Results

Results are automatically calculated by the VITROS Immunodiagnostic and VITROS Integrated Systems.

Result Calculation

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



Interpretation of Results

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

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

A sample found "Retest?" or "Reactive" should be retested in duplicate to verify its status. If results on repeat testing are <0.90 for both replicates, the sample should be considered negative. If either retest result is ≥1.00, the sample should be considered Reactive and tested by supplemental tests to confirm the result. A repeatedly reactive sample, confirmed by supplemental tests must be considered positive for anti-HIV-1 (including Group O), and/or anti HIV-2 antibody and/or p24 antigen. In the case of repeatedly "Retest?" results, analysis of follow-up samples is recommended.

The following table summarizes the interpretation of results obtained with the VITROS HIV Combo test on the VITROS Immunodiagnostic and VITROS Integrated Systems.

VITROS HIV Combo Test Result (s/c)	Action	Interpretation
<0.90	No further testing	Specimen is negative for anti-HIV-1 (including Group O), anti-HIV-2 and p24 antigen.
≥0.90 and <1.00	Retest in duplicate	Specimen is negative for anti-HIV-1 (including Group O), anti-HIV-2 and p24 antigen if both duplicate results are <1.00 s/c. Specimen is reactive for anti-HIV-1 (including Group O), and/or anti-HIV-2 and/or p24 antigen if 1 or both duplicate results are ≥1.00 s/c.
≥1.00	Retest in duplicate	Specimen is reactive for anti-HIV-1(including Group O), and/or anti-HIV-2 and or p24 antigen if 1 or both duplicate results are ≥1.00 s/c.

- If a specimen is reactive the probability that HIV antibodies or antigen are present is high, especially in subjects at high risk for HIV infection. After confirming the HIV Combo test result by repeating in duplicate, it is appropriate to investigate reactive results by additional, more specific tests. Specimens 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 HIV infection is accurate.
- Interpretation of results from specimens 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 testing another specimen obtained three to six months later.
- The magnitude of a VITROS HIV Combo test result cannot be correlated to an endpoint titre.

Limitations of the Procedure

Known Interferences

The VITROS HIV Combo test was evaluated for interference consistent with CLSI document EP7. [8] 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. [9] These antibodies may be present in blood samples from individuals regularly exposed to animals or who have been treated with animal serum products. Results which are inconsistent with clinical observations indicate the need for additional testing.
- Certain drugs and clinical conditions are known to alter antibody concentrations *in vivo*. For additional information, refer to one of the published summaries. [10] [11] [12]
- Do not use quality control materials preserved with azide.

Performance Characteristics

Clinical Performance

Sensitivity

500 patient samples previously determined as positive were tested in the VITROS HIV Combo test.

	Number Tested	Number Reactive
HIV-1	400	400
HIV-2	100	100
Total	500	500

The sensitivity for this population of samples in the VITROS HIV Combo test was calculated as 100.00% (500/500) with an exact 95% confidence interval of 99.26% to 100.00%.

In addition, 83 samples known to be infected with HIV-1 group M subtypes were reactive by the VITROS HIV Combo test.

Antibody Subtype	Number Tested	VITROS HIV Combo Number Reactive
A	5	5
A1	1	1
A2	2	2

Antibody Subtype	Number Tested	VITROS HIV Combo Number Reactive
B	2	2
C	7	7
D	4	4
F	2	2
F1	2	2
F2	5	5
G	7	7
H	3	3
H/A1	1	1
H/U	1	1
J	4	4
K	3	3
CRF01	2	2
CRF01/AE	5	5
CRF01/CRF15	1	1
CRF02/AG	11	11
CRF02/G	2	2
CRF06	1	1
CRF06/CPX	2	2
CRF09/K	1	1
CRF09/CPX	3	3
CRF11/CPX	2	2
CRF18/CPX	4	4
Total	**83**	**83**

Antigen Positive Sample Sensitivity

Fifty one HIV antigen positive samples were tested and shown to be reactive in the VITROS HIV Combo Test.

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 a commercially available CE marked 4th generation Ag/Ab combo test is presented in the table below.

Source (# of Specimens)	Subtype	Number of Specimens Tested	VITROS HIV Combo Test	Commercially Available 4th Generation HIV Ag/Ab 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)	7 (87.5)
Djibouti (1), Ethiopia (1), Senegal (1), Somalia (1), Uganda (1), Zambia (1), Unknown (1)	C	7	5 (71.4)	5 (71.4)
Senegal (1), Uganda (2)	D	3	3 (100)	3 (100)
Brazil (3), Romania (2)	F	5	5 (100)	5 (100)
Kenya (1), Democratic Republic of the Congo (1)	G	2	2 (100)	1 (50.0)
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)	47 (90.4)

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

Detection of HIV-1 p24 Antigen Standards

Sensitivity was determined by testing serial dilutions of the NIBSC HIV-1 p24 Antigen Standard (90/636) and the AFSSAPS HIV-1 p24 Antigen Standard in three determinations using two reagent lots across all the VITROS Immunodiagnostic and Integrated Systems. The overall sensitivity of the VITROS HIV Combo test for the NIBSC HIV-1 p24 Antigen Standard (90/636) was ≤0.48 IU/mL. The overall sensitivity of the VITROS HIV Combo test for the AFSSAPS HIV-1 p24 Antigen Standard was ≤13.1 pg/mL.

Seroconversion Panels

Thirty four commercially available seroconversion panels were tested on both the VITROS HIV Combo Test and a commercially available CE-marked 4th generation Ag/Ab combo test. Results for the thirty four panels are summarized in the following table. The table presents the number of reactive panel members, the days from first bleed to first reactive result and the difference in days to first reactive between the two tests.

Days to Evidence of HIV Infection

Panel ID	Number of Reactive Panel Members		Days to First Reactive Result		Difference in Days to First Reactive Result *
	VITROS HIV Combo Test	Commercially Available 4th Generation HIV Ag/Ab Test	VITROS HIV Combo Test	Commercially Available 4th Generation HIV Ag/Ab Test	
PRB934	3	3	0	0	0
PRB950	3	2	18	21	3
PRB954	2	2	17	17	0
PRB966	3	3	44	44	0
6243	4	4	25	25	0
6244	2	2	27	27	0
6247	4	4	21	21	0
6248	2	2	18	18	0
9021	4	4	47	47	0
9079	17	17	40	40	0

Panel ID	Number of Reactive Panel Members		Days to First Reactive Result		Difference in Days to First Reactive Result *
	VITROS HIV Combo Test	Commercially Available 4th Generation HIV Ag/Ab Test	VITROS HIV Combo Test	Commercially Available 4th Generation HIV Ag/Ab Test	
HIV9012	4	3	14	16	2
HIV9014	6	6	0	0	0
HIV9077	16	16	42	42	0
HIV9020	3	3	89	89	0
HIV9018	4	3	25	28	3
HIV9015	2	2	30	30	0
PRB955	4	4	3	3	0
PRB930	4	4	0	0	0
PRB951	4	4	8	8	0
PRB963	2	2	17	17	0
HIV12007	6	6	117	117	0
HIV12008	6	5	23	28	5
HIV9013	1	1	25	25	0
HIV9028	2	2	53	53	0
HIV9032	8	7	22	24	2
HIV9075	3	3	22	22	0
HIV9089	3	3	16	16	0
PRB943	5	5	7	7	0
PRB956	2	2	47	47	0
PRB957	2	3	23	16	–7
PRB960	2	2	28	28	0
PRB961	2	2	27	27	0
PRB962	2	2	14	14	0
PRB964	1	1	22	22	0
Total	138	134	931	939	8

* Days to first reactive test result on the commercially available test minus the days to first reactive test result for the VITROS HIV Combo test

The VITROS HIV Combo Test and the commercially available 4th generation Ag/Ab Combo Test were in agreement for 28 of the 34 panels. The VITROS HIV Combo Test became reactive one bleed earlier for five of the thirty four panels. The commercially available 4th generation Ag/Ab Combo Test became reactive one bleed earlier for one panel.

Specificity

Samples from 5077 presumed healthy blood donors, and 608 clinical specimens were tested at two external sites in the VITROS HIV Combo test and another commercially available CE marked 4th generation Ag/Ab Combo Test.

Samples	Number of test samples	Initially Reactive	Repeatedly Reactive	Confirmed Reactive
Donor	5077	16	8	0
Clinical	608	1	1	1*

* Sample confirmed as Reactive in a 3rd generation antibody immunoassay, a line immunoassay and a nucleic acid test (NAT). This sample was excluded from the calculation of specificity.

The specificity of the VITROS HIV Combo test for the donor population was calculated as 99.84% (5069/5077) exact 95% CI (99.69-99.93%). The specificity of the VITROS HIV Combo test for the clinical population was calculated as 100.00% (607/607) exact 95% CI (99.39-100.00%).

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.

Summary of VITROS HIV Combo Test Results with Potentially Cross-Reacting Samples

Sample Category	Number Tested	Number Negative	Number Reactive
HCV Antigen	6	6	0
HCV Antibody	10	10	0
HBsAg	6	6	0

Sample Category	Number Tested	Number Negative	Number Reactive
HBc Antibody	10	10	0
HTLV I Antigen	6	6	0
HTLV II Antigen	6	6	0
HTLV I Antibody	6	6	0
HTLV II Antibody	6	6	0
EBV Antigen	6	6	0
EBV Antibody	10	10	0
HSV I Antigen	6	6	0
HSV II Antigen	6	6	0
HSV Antibody	12	12	0
Chlamydia	10	10	0
Gonorrhea	10	10	0
Syphilis	10	10	0
Multiparous Female	10	10	0
Pregnancy (1st Trimester)	8	8	0
Pregnancy (2nd Trimester)	8	8	0
Pregnancy (3rd Trimester)	8	8	0
Pre Flu Vaccine	5	5	0
Post Flu Vaccine	5	5	0
Influenza A Antigen	6	6	0
Influenza B Antigen	6	6	0
Rheumatoid Factor (RF)	8	8	0
Human Anti-Mouse Antibody (HAMA)	10	10	0
Autoimmune Disease	10	10	0
Anti-Nuclear Antibodies (ANA)	10	10	0
Hemophilia	11	11	0
Dialysis	10	10	0
Yeast Reactive: Candida	10	10	0
Super-oxide Dismutase (SOD)	1	1	0
Cytomegalovirus Antigen	6	6	0
Cytomegalovirus Antibody	10	10	0
Toxoplasmosis infection	10	10	0
HAV Antigen	6	6	0
HAV Antibody	10	10	0
Rubella infection	10	10	0
Elevated IgG	11	11	0
Elevated IgM	7	7	0
Non-Viral Liver Disease	10	10	0
Pediatric (2-5 yrs)	4	4	0
Pediatric (6-10 yrs)	6	6	0
Pediatric (11-16 yrs)	6	6	0
Pediatric (17-21 yrs)	4	4	0
Elevated Cholesterol	10	10	0
Elevated Total Protein	9	9	0
Elevated Triglycerides	6	6	0

Precision

Precision was evaluated consistent with CLSI dcument EP5. [13] Two replicates each of 14 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 2 reagent lots on 2 different systems on the VITROS ECi/ECiQ Immunodiagnostic

System, VITROS 3600 Immunodiagnostic System and VITROS 5600 Integrated System. The data presented are a representation of the product performance on each system.

	VITROS 3600 Immunodiagnostic System								
		Within-run*		Within-calibration**		Within-lab***		No.	
Panel Member	Mean (S/C)	SD	CV (%)	SD	CV (%)	SD	CV (%)	Observ.	No. Days
Negative	0.06	0.010	N/A	0.013	N/A	0.013	N/A	88	22
Negative	0.07	0.008	N/A	0.011	N/A	0.012	N/A	88	22
Negative Control	0.07	0.009	N/A	0.014	N/A	0.014	N/A	88	22
Anti-HIV-1	0.56	0.024	4.4	0.051	9.3	0.053	9.3	88	22
Anti-HIV-1	0.98	0.031	3.2	0.076	7.8	0.076	7.6	88	22
Anti-HIV-1	2.17	0.057	2.7	0.121	5.7	0.119	5.4	88	22
Anti-HIV-1 Reactive Control	1.90	0.071	3.8	0.115	6.1	0.116	6.0	88	22
Anti-HIV-2	0.72	0.036	5.1	0.072	10.1	0.073	10.0	88	22
Anti-HIV-2	1.04	0.038	3.7	0.075	7.3	0.074	7.0	88	22
Anti-HIV-2	2.44	0.075	3.1	0.129	5.4	0.126	5.1	88	22
Anti-HIV-2 Reactive Control	4.20	0.124	3.0	0.190	4.6	0.190	4.5	88	22
Anti-HIV-1 Group O	0.86	0.044	5.2	0.074	8.7	0.076	8.7	88	22
Anti-HIV-1 Group O	1.10	0.046	4.2	0.080	7.3	0.081	7.3	88	22
Anti-HIV-1 Group O	2.38	0.093	4.0	0.131	5.6	0.136	5.6	88	22
Anti-HIV-1 Group O Reactive Control	3.30	0.106	3.3	0.172	5.3	0.167	5.0	88	22
HIV p24 Ag	0.78	0.018	2.3	0.057	7.4	0.059	7.5	88	22
HIV p24 Ag	1.40	0.028	2.0	0.073	5.3	0.077	5.4	88	22
HIV p24 Ag	3.33	0.049	1.5	0.108	3.3	0.116	3.4	88	22
HIV p24 Ag Reactive Control	1.92	0.033	1.7	0.089	4.7	0.092	4.7	88	22

* 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.

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

N/A = Not applicable

Panel Member	VITROS 5600 Integrated System**** Mean (S/C)	Within-run* SD	Within-run* CV (%)	Within-calibration** SD	Within-calibration** CV (%)	Within-lab*** SD	Within-lab*** CV (%)	No. Observ.	No. Days
Negative[b]	0.05	0.007	N/A	0.009	N/A	0.009	N/A	84	21
Negative[c]	0.07	0.008	N/A	0.009	N/A	0.009	N/A	84	21
Negative Control	0.07	0.014	N/A	0.015	N/A	0.015	N/A	88	22
Anti-HIV-1	0.55	0.024	4.4	0.041	7.6	0.042	7.6	88	22
Anti-HIV-1	0.97	0.033	3.4	0.057	5.9	0.058	6.0	88	22
Anti-HIV-1	2.17	0.048	2.2	0.100	4.6	0.099	4.5	88	22
Anti-HIV-1 Reactive Control	1.90	0.056	3.0	0.093	4.9	0.092	4.8	88	22
Anti-HIV-2	0.70	0.038	5.5	0.061	8.8	0.060	8.6	88	22
Anti-HIV-2	1.03	0.043	4.2	0.061	6.0	0.061	5.9	88	22
Anti-HIV-2	2.45	0.069	2.8	0.124	5.1	0.125	5.1	88	22
Anti-HIV-2 Reactive Control	4.26	0.123	2.9	0.156	3.7	0.156	3.6	88	22
Anti-HIV-1 Group O	0.84	0.032	3.9	0.061	7.3	0.061	7.3	88	22
Anti-HIV-1 Group O	1.09	0.036	3.3	0.067	6.2	0.067	6.1	88	22
Anti-HIV-1 Group O	2.37	0.095	4.0	0.133	5.6	0.143	6.0	88	22
Anti-HIV-1 Group O Reactive Control	3.38	0.100	3.0	0.131	3.9	0.134	3.9	88	22
HIV p24 Ag	0.80	0.021	2.7	0.046	5.8	0.046	5.8	88	22
HIV p24 Ag	1.47	0.035	2.4	0.066	4.5	0.068	4.6	88	22
HIV p24 Ag	3.52	0.057	1.6	0.099	2.8	0.115	3.2	88	22
HIV p24 Ag Reactive Control	2.03	0.037	1.8	0.072	3.6	0.076	3.7	88	22

* 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 from analysis due to a statistical outlier (1.42 S/C)

[c] One test day removed from analysis due to a statistical outlier (1.15 S/C)

N/A = Not applicable

Panel Member	VITROS ECi/ECiQ Immunodiagnostic System							No. Observ.	No. Days
	Mean (S/C)	Within-run*		Within-calibration**		Within-lab***			
		SD	CV (%)	SD	CV (%)	SD	CV (%)		
Negative[b]	0.05	0.004	N/A	0.005	N/A	0.005	N/A	84	21
Negative	0.07	0.005	N/A	0.007	N/A	0.007	N/A	88	22
Negative Control	0.07	0.024	N/A	0.024	N/A	0.025	N/A	88	22
Anti-HIV-1	0.65	0.034	5.2	0.045	6.9	0.046	7.1	88	22
Anti-HIV-1	1.09	0.088	8.1	0.106	9.8	0.105	9.6	88	22
Anti-HIV-1	2.38	0.095	4.0	0.122	5.1	0.122	5.1	88	22
Anti-HIV-1 Reactive Control	1.96	0.070	3.6	0.113	5.8	0.115	5.8	88	22
Anti-HIV-2	0.72	0.030	4.2	0.045	6.3	0.048	6.7	88	22
Anti-HIV-2	1.06	0.045	4.3	0.070	6.7	0.073	6.9	88	22
Anti-HIV-2	2.43	0.076	3.1	0.130	5.4	0.142	5.8	88	22
Anti-HIV-2 Reactive Control	4.11	0.175	4.3	0.196	4.8	0.204	5.0	88	22
Anti-HIV-1 Group O	0.96	0.056	5.9	0.073	7.7	0.070	7.3	88	22
Anti-HIV-1 Group O	1.22	0.048	4.0	0.074	6.1	0.077	6.3	88	22
Anti-HIV-1 Group O	2.61	0.168	6.5	0.190	7.3	0.190	7.3	88	22
Anti-HIV-1 Group O Reactive Control	3.49	0.093	2.7	0.139	4.0	0.153	4.4	88	22
HIV p24 Ag	0.93	0.026	2.8	0.045	4.9	0.049	5.3	88	22
HIV p24 Ag	1.65	0.041	2.5	0.069	4.2	0.070	4.2	88	22
HIV p24 Ag	3.87	0.057	1.5	0.120	3.1	0.137	3.5	88	22
HIV p24 Ag Reactive Control	2.29	0.038	1.7	0.066	2.9	0.079	3.4	88	22

* 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.

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

[b] One test day removed from analysis due to a statistical outlier (1.90 S/C)

N/A = Not applicable

Specificity

Substances that do not Interfere

The VITROS HIV Combo test was evaluated for interference consistent with CLSI document EP7.[8] 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	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	N/A
IgG	1680 mg/dL	16.80 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

N/A = Not applicable (alternate units are not provided)

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.

Do Not Reuse	Upper Limit of Temperature	Range
Use by or Expiration Date (Year-Month-Day)	Lower Limit of Temperature	Range of Means
LOT Batch Code or Lot Number	Temperature Limitation	Midpoint
SN Serial Number	Consult Instructions for Use	Revised
REF Catalog Number or Product Code	Attention: The Instructions for Use (IFU) has been updated	Supersedes
Caution	S1 For use in Slide Supply 1	Contains Sufficient for "n" Tests
Keep Dry (Protect from Moisture/Humidity)	S2 For use in Slide Supply 2	IVD *in vitro* Diagnostic Medical Device
Manufacturer	SI SI Units	Der Grüne Punkt (the Green Dot). Manufacturer follows certain packaging material waste disposal management regulations
Date of Manufacture	CONV Conventional Units	Estimated within-lab SD
EC REP Authorized Representative in the European Community	Value	
Corrosive	Flammable	Serious Health Hazards
Health Hazards	Acute Toxicity	Environmental or Aquatic Toxicity

Revision History

Date of Revision	Version	Description of Technical Changes*
2019-11-19	7.1	Clinical Performance: Seroconversion Panels: corrected data
2019-09-06	7.0	• Clinical Performance: Seroconversion Panels: corrected data • Glossary of Symbols: updated • Added EC Representative address

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

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