HUMAN IMMUNODEFICIENCY VIRUS TYPES 1 and 2

(Recombinant and Synthetic Peptides)

GS HIV-1/HIV-2 *PLUS O* EIA

Recombinant and Synthetic Peptide Enzyme Immunoassay (EIA) for the Detection of Antibody to Human Immunodeficiency Virus Types 1 (Groups M and O) and/or 2 (HIV-1/HIV-2) in Human Serum, Plasma, or Cadaveric Serum Specimens

For In Vitro Diagnostic Use

32588 • 480 Tests
32589 • 960 Tests
25256 • 4800 Tests

LEXICON	
IVD	For In Vitro Diagnostic Use
CE	European Conformity
REF	Catalog Number
	Consult Instructions for Use
	Number of Tests
	Temperature Limit
	Manufactured by
EC REP	Authorized Representative in the European Community
WASH	Wash Solution Concentrate (30X)
TMB SOLUTION	Chromogen: TMB Solution
SUB BUF	Substrate Buffer
STOP	Stopping Solution
	Biohazard
	WARNING
	Corrosive

CONTENTS

1 - NAME AND INTENDED USE

The GS HIV-1/HIV-2 *PLUS O* EIA is an enzyme immunoassay utilizing recombinant proteins and synthetic peptides for the detection of antibodies to HIV-1 (Groups M and O) and/or HIV-2 in human serum and plasma. It is indicated as a screening test for specimens from individual human donors, including donors of whole blood, blood components, and source plasma, and from other living donors. It is also intended for use in testing plasma and serum specimens to screen organ donors when specimens are obtained while the donor's heart is still beating, and in testing blood specimens from cadaveric (non-heartbeating) donors. In addition, it is intended as an aid in the diagnosis of infection with HIV-1 and/or HIV-2. The assay is not intended for use on cord blood specimens.

The GS HIV-1/HIV-2 *PLUS O* EIA is intended for manual use and also for use with the ORTHO® Summit System (OSS) in the screening of blood donors.

The GS HIV-1/HIV-2 *PLUS O* EIA is intended for manual use and also for use with the EVOLIS® and Elite™ Automated Microplate Systems as an aid in the diagnosis of infection with HIV-1 and/or HIV-2.

2 - SUMMARY AND EXPLANATION OF THE TEST

The acquired immunodeficiency syndrome (AIDS) is caused by viruses transmitted by sexual contact, exposure to blood (including sharing contaminated needles and syringes) or certain blood products, or transmitted from an infected mother to her fetus or child during the perinatal period.[1] Additionally, transmission of these viruses can occur through tissue transplantation.[2] Human Immunodeficiency Virus Type 1 (HIV-1) has been isolated from patients with AIDS and AIDS-related complex (ARC).[3-5] HIV-1 was thought to be the sole causative agent of these syndromes until 1986, when a second type of Human Immunodeficiency Virus (Human Immunodeficiency Virus Type 2 or HIV-2) was isolated and also reported to cause

AIDS.[6-7] Since the initial discovery, hundreds of cases of HIV-2 infection have been documented worldwide, including cases of AIDS related to HIV-2.[8] In the United States, there have been more than 80 cases of infection with HIV-2 reported, including three potential blood donors.[9-14]

This second immunodeficiency virus is similar to, but distinct from, HIV-1. Both viruses have similar morphology and lymphotropism,[15] and the modes of transmission appear to be identical.[8,16] The HIV-1 and HIV-2 genomes exhibit about 60% homology in conserved genes such as gag and pol, and 39-45% homology in the envelope genes.[17] Serologic studies have also shown that the core proteins of HIV-1 and HIV-2 display frequent cross-reactivity, whereas the envelope proteins are more type-specific.[18]

Within the two major HIV types, there is significant variation as well. By analyzing sequences of representative strains, HIV-1 has been divided into three groups: Group M (for major), including at least ten subtypes (A through J); Group O (for outlier); and Group N (for non-M, non-O).[19-21] Similarly, the HIV-2 strains have been classified into at least five subtypes (A through E).[22] Some HIV-1 variants share $\leq$ 50% homology in their envelope genes with the sequences of more common prototype strains.

Despite some degree of immunological cross-reactivity between types and subtypes of HIV, reliable detection of the more divergent strains may only be achieved by incorporating specific sequences into the assay design. In one study, detection of HIV-2 positive samples by licensed HIV-1 antibody kits ranged from 60% to 91%, depending on the test used.[23] Detection of HIV-1 Group O samples by HIV-1 and HIV-1/HIV-2 assays varied from 0% to 100% in studies with U.S.-licensed and European test kits.[24,25] The GS HIV-1/HIV-2 *PLUS O* EIA incorporates highly conserved recombinant and synthetic peptide sequences representing HIV-1 (Groups M and O) and HIV-2.[26-32] It was developed to improve sensitivity and specificity of detection of

antibodies to HIV-1 and/or HIV-2 for blood and plasma screening, and as an aid in the diagnosis of HIV infection.

Any specimen that reacts in an initial test (is initially reactive) with the GS HIV-1/HIV-2 *PLUS O* EIA must be retested in duplicate with the GS HIV-1/HIV-2 *PLUS O* EIA. Initially reactive specimens that are reactive in either one or both duplicates from the repeat testing are referred to as repeatedly reactive. Repeatedly reactive specimens may contain antibodies to either HIV-1 or HIV-2. Therefore, additional, more specific or supplemental tests for antibodies to both HIV-1 and HIV-2, such as Western blot or immunofluorescence, must be performed to verify the presence of antibodies to HIV-1 or HIV-2. Recommendations for appropriate use of such additional tests may be issued periodically by the United States Public Health Service.

3 - BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The GS HIV-1/HIV-2 *PLUS O* EIA is an enzyme immunoassay based on the principle of the direct antibody sandwich technique. Microwell strip plates (the solid phase) are coated with purified antigens: gp160 and p24 recombinant proteins derived from HIV-1; a peptide representing the immunodominant region of the HIV-2 transmembrane glycoprotein, gp36; and a synthetic polypeptide mimicking an artificial (i.e., encoded by no existing virus) HIV-1 Group O specific epitope.

Samples and controls are added to the wells along with Specimen Diluent. The Specimen Diluent contains a dye that changes color from purple to blue when combined with a sample or control. The wells are incubated and then washed. The next step is the addition of a colored Conjugate Solution (green), which contains peroxidase-conjugated antigens (peptides mimicking various immunodominant epitopes of the HIV-1 and HIV-2 transmembrane glycoproteins, and a p24 recombinant protein). The wells are then incubated. If HIV-1 and/or HIV-2 antibody is present, it will bind to the antigen coated on the well and to the peroxidase-conjugated antigens in the Conjugate. The antigen-antibody-antigen complexes remain bound to the

well during the subsequent wash step, which will remove any unbound materials. Working TMB Solution is added to the plate and allowed to incubate. A blue or blue-green color develops in proportion to the amount of HIV antibody present in the sample. Color development is stopped by the addition of acid, which changes the blue-green color to yellow. The optical absorbance of specimens and controls is determined spectrophotometrically at a wavelength of 450 nm.

4 - REAGENTS

GS HIV-1/HIV-2 *PLUS O* EIA Product Description

Product No. 32588 (480 Tests), 32589 (960 Tests), 25256 (4800 Tests)

Component	Contents	Preparation
R1 • HIV-1/HIV-2 *PLUS O* Microwell Strip Plate 5, 10, or 50	• Microwell plate with adsorbed purified HIV-1 and HIV-2 antigens. • ProClin 150 preservative • Tabs are labeled "BB"	Use as supplied. Return unused strips/plates to pouch and reseal. Do not remove desiccant.
R2 • Wash Solution Concentrate (30X) 2, 3, or ** bottles (120 mL)	• Sodium chloride • Tween 20™	Dilute 1:30 with deionized water. Clinical laboratory reagent water (CLRW) is acceptable.
R3 • Specimen Diluent 1, 1, or 5 bottle(s) (100 mL)	• Bovine proteins • Buffer with protein stabilizers • 0.1% ProClin 300 preservative • Sample indicator dye	Use as supplied.
C0 • Negative Control 1, 1, or 5 vial(s) (8 mL)	• Human serum; negative for HIV and HCV antibodies and HBsAg • 0.005% Gentamicin sulfate • 0.16% ProClin 950 preservative	Dilute in Specimen Diluent as described.
C1 • HIV-1 Positive Control 1, 2, or 10 vial(s) (1.4 mL)	• Human HIV-1 antibody in human serum/plasma • Non-reactive for HBsAg and antibodies to HCV • 0.005% Gentamicin sulfate • 0.16% ProClin 950 preservative	Dilute in Specimen Diluent as described.
C2 • HIV-2 Positive Control 1, 2, or 10 vial(s) (1.4 mL)	• Human HIV-2 antibody in human serum/plasma • Non-reactive for HBsAg and antibodies to HCV • 0.005% Gentamicin sulfate • 0.16% ProClin 950 preservative	Dilute in Specimen Diluent as described.
C3 • HIV-1 Group O Positive Control 1, 2, or 10 vial(s) (1.4 mL)	• Rabbit HIV-1 Group O antibody in human serum, negative for HIV and HCV antibodies and HBsAg • 0.005% Gentamicin sulfate • 0.16% ProClin 950 preservative	Dilute in Specimen Diluent as described.

GS HIV-1/HIV-2 *PLUS O* EIA Product Description(*cont.*)

Component	Contents	Preparation
R4 • Conjugate Concentrate (11X) 1, 1, or 5 vial(s), (12 mL)	• Purified HIV-1 and HIV-2 antigens labeled with peroxidase • Buffer with protein stabilizers (bovine and caprine) • 0.005% Gentamicin sulfate • 0.5% ProClin 300 preservative • Green dye	Dilute in Conjugate Diluent as described.
R5 • Conjugate Diluent 1, 1, or 5 bottle(s), (120 mL)	• Buffer with protein stabilizers (bovine) • 0.1% ProClin 300 preservative	Use as supplied.
R8 • Substrate Buffer 1, 1, or 5 bottle(s) (120 mL)	• Hydrogen peroxide • Citric acid/Sodium acetate buffer • DMSO	Use as supplied.
R9 • Chromogen (11X) 1, 1, or 5 bottles(s) (12 mL)	• Tetramethylbenzidine (TMB)*	Dilute with Substrate Buffer as described.
R10 • Stopping Solution 1, 1, or ** bottle(s), (120 mL)	• 1N Sulfuric acid (H_2SO_4)	Use as supplied.

* Note: Tetramethylbenzidine is a non-carcinogenic and non-mutagenic chromogen for peroxidase.[33,34]

** Wash Solution Concentrate and Stopping Solution must be purchased separately for the 50 plate (4800 test) kit. Refer to catalog number 25261 for the Wash Solution Concentrate and catalog number 25260 for the Stopping Solution. These reagents are included in the 5 plate (480 test) and 10 plate (960 test) kits.

Store kit at 2-8°C. Bring all reagents except Conjugate Concentrate to room temperature (18-30°C) before use. Return reagents to 2-8°C after use. Return unused strips/plates to pouch and reseal. Do not remove desiccant. Store strips/plates at 2-8°C.

5 - WARNINGS FOR USERS

For In Vitro Diagnostic Use

WARNING: FDA has licensed this test for use with serum, plasma, and cadaveric serum specimens only. Use of this licensed test kit with specimens other than those specifically approved for use with this test kit may cause inaccurate test results.

1. This test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with their potential hazards. Handle appropriately with the requisite Good Laboratory Practices. Wear appropriate protective clothing, including lab coat, eye/face protection, and disposable gloves (synthetic, non-latex gloves are recommended) while handling kit reagents and clinical

specimens. Wash hands thoroughly after performing the test.

2. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
3. Do not pipette by mouth.
4. The following is a list of potential chemical hazards contained in some reagents (refer to Product Description chart):
   a. 0.005% Gentamicin Sulfate, a biocidal preservative, which is a known reproductive toxin, photosensitizer, and sensitizer; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.
   b. **WARNING:** Components R3, R4 and R5 contain **0.1% or 0.5% ProClin 300**.

H317: May cause 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.

P501: Dispose of contents and container in accordance with local, regional, national, and international regulations.

ProClin 300 (0.1% or 0.5%) is a biocidal preservative that is irritating to eyes and skin, may be detrimental if enough is ingested, and may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.

c. **DANGER!** The **Stopping Solution** (R10) contains **1N Sulfuric Acid**.

H314: Causes severe skin burns and eye damage.

H290: May be corrosive to metals.

P280: Wear protective gloves/protective clothing/eye protection/face protection.

P301 + P330 + P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.

P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P501: Dispose of contents and container in accordance with local, regional, national, and international regulations.

The 1N Sulfuric Acid (H_2SO_4) Stopping Solution is severely irritating or corrosive to eyes and skin, depending on the amount and length of exposure; greater exposures can cause eye damage, including permanent impairment of vision. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Keep away from strong bases, reducing agents, and metals; do not pour water into this component. Waste from this material is considered hazardous acidic waste, however if permitted by local, regional, and national regulations, it might be neutralized to pH 6-8 for non-hazardous disposal.

5. The GS HIV-1/HIV-2 *PLUS O* EIA contains human blood components. No known test method can offer complete assurance that infectious agents are absent. Therefore, all human blood derivatives, reagents, and human specimens

should be handled as if capable of transmitting infectious disease, following recommended *Standard and Universal Precautions* for bloodborne pathogens as defined by OSHA, Biosafety Level 2 guidelines from the current CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories*[35], WHO *Laboratory Biosafety Manual*[36], and/or local, regional, and national regulations. The following human blood derivatives are found in this kit:

a. Human source material used in the preparation of the Negative Control (C0) and as a diluent for the Positive Controls (C1, C2, and C3) has been tested and found non-reactive for Hepatitis B surface antigen (HBsAg), and antibodies to Hepatitis C virus (HCV Ab) and human immunodeficiency virus (HIV-1 and HIV-2).

b. Human source material, containing HIV-1 and HIV-2 human antibody used in the preparation of the Positive Controls (C1 and C2) has been heat-treated. It has been tested and found nonreactive for Hepatitis B surface antigen (HBsAg) and antibodies to Hepatitis C virus (HCV Ab).

6. Biological spills: Human source material spills should be treated as potentially infectious.

   Spills not containing acid should be immediately decontaminated, including the spill area, materials, and any contaminated surfaces or equipment, with an appropriate chemical disinfectant that is effective for the potential biohazards relative to the samples involved (commonly a 1:10 dilution of household bleach, 70-80% Ethanol or Isopropanol, an iodophor [such as 0.5% Wescodyne Plus, EPA Registration #4959-16-52], or a phenolic), and wiped dry.[37-40]

   Spills containing acid should be appropriately absorbed (wiped up) or neutralized, and then the area wiped with one of the chemical disinfectants. Material used to absorb the spill may require biohazardous waste disposal.

NOTE: DO NOT PLACE SOLUTIONS CONTAINING BLEACH INTO THE AUTOCLAVE.

7. Dispose of all specimens and material used to perform the test as though they contain an infectious agent. Laboratory, chemical, or biohazardous wastes must be handled and discarded in accordance with all local, regional, and national regulations.
8. There are no health hazards associated with the intact desiccant packet; do not cut, split, or otherwise compromise it as dusts that may be generated could pose a health hazard. If the desiccant has been compromised, do not remove it from the plate pouch.
9. Complete hazard information and precautions are located in the Safety Data Sheet (SDS) available at bio-rad.com or upon request from Bio-Rad Technical Services.

6 - PRECAUTIONS FOR USERS

1. Do not use any kit components beyond their stated expiration date.
2. The reagents that may be used with different lots of the GS HIV-1/HIV-2 *PLUS O* EIA kit are the Chromogen (R9), Substrate Buffer (R8), Wash Solution Concentrate (R2), and Stopping Solution (R10). Do not mix any other reagents from different lots. Any lot number of the following reagents may be used with this assay provided they have the correct catalog number and are not used beyond their labeled expiration date:
   - Chromogen (R9) - Catalog #26182
   - Substrate Buffer (R8) - Catalog #26181
   - Wash Solution Concentrate (R2) - Catalog #25261
   - Stopping Solution (R10) - Catalog #25260
3. The tabs at the end of the microwell strips are labeled with product code “BB.” Do not use strips that have other product codes with this kit.

4. Exercise care when opening vials and removing aliquots to avoid microbial contamination of the reagents.
5. Use a clean, disposable container for the Conjugate Solution. Exposure of the Conjugate to sodium azide will result in its inactivation.
6. Avoid exposing Chromogen or the Working TMB Solution to strong light during storage or incubation. Do not allow the chromogen solutions to come into contact with an oxidizing agent.
7. Use clean, **polypropylene** containers **(do not use polystyrene containers)** to prepare and store the Working TMB Solution. If glassware must be used, pre-rinse thoroughly with 1N sulfuric or hydrochloric acid followed by at least three washes of deionized water. Be sure that no acid residue remains on the glassware. If polypropylene containers are to be reused, they should be cleaned in accordance with a cleaning process validated by the testing facility.
8. Bring all reagents except Conjugate Concentrate to room temperature before use.
9. Clinical samples may contain very high levels of HIV antibody. Therefore, care must be exercised when dispensing samples to avoid cross contamination through aerosols or carryover. For manual pipetting of controls and specimens, use an individual pipette tip for each sample and do not allow other parts of the pipetting device to touch the rim or interior of the specimen container. Consider using new stoppers/caps to seal specimen tubes after use, to avoid errors or contamination of the work area while recapping tubes.
10. Handle the Negative Control and Positive Controls in the same manner as patient specimens.

11. If a specimen or reagent is inadvertently not added to a well, the assay results will read negative. Reagents of this kit have been color-coded to enable confirmation of the addition of specimens/controls and Working Conjugate Solution.
12. Inadequate adherence to package insert instructions may cause erroneous results.
13. Use only adequately calibrated equipment with this assay.
14. Use of dedicated equipment is recommended if equipment performance validations have not precluded the possibility of cross-contamination.
15. The GS HIV-1/HIV-2 *PLUS O* EIA performance is highly dependent upon incubation times and temperatures and effective washing. Temperatures outside of the validated ranges may result in invalid assays. Incubation temperatures should be carefully monitored using calibrated thermometers, or equivalent.
16. **Caution: Certain washer conditions, such as partially blocked cannulae that are not detected by the OSP instrument, can lead to sub-optimal washing and false reactive test results. It is recommended that users of ORTHO® Summit System carefully verify that the washing system is clear and operating properly before performing an assay.**

7 - REAGENT PREPARATION AND STORAGE

Working Conjugate Solution

Note: 1:11 dilution. Bring Conjugate Diluent (R5) to room temperature. **Use only the matched lot of Conjugate Concentrate provided with the kit master lot being used.** (See PRECAUTIONS FOR USERS section, item 2, page 12.)

Invert Diluent (colorless to pale straw) and Conjugate Concentrate (R4, green) to mix before using. Prepare a 1:11 dilution for each strip to be tested by adding 100 µL of Conjugate Concentrate to 1 mL of Conjugate Diluent in a clean,

plastic tube. Use the following table as a guide. Ensure that the volume of Working Conjugate Solution that is prepared will be adequate for the entire run. Mix well. Working Conjugate Solution should be green. Note Concentrate lot number, date and time of preparation, and date and time of expiration of the Working Conjugate Solution. Working Conjugate Solution is stable for 8 hours at room temperature. Mix working solution prior to use.

Return Conjugate Concentrate to the refrigerator immediately after use. To avoid contamination of Conjugate, wear clean gloves and do not touch tips of pipettes.

Preparation of Working Conjugate Solution by Strip

Number of Strips to be used	1	2	3	4	5	6	7	8	9	10	11	12*
Amount of Conjugate Concentrate (µL)	100	200	300	400	500	600	700	800	900	1000	1100	1200
Amount of Conjugate Diluent (mL)	1	2	3	4	5	6	7	8	9	10	11	12

* Complete Plate

Preparation of Working Conjugate Solution by Plate

Number of Complete Plates to be used	1	2	3	4	5	6	7	8	9	10
Amount of Conjugate Concentrate (mL)	1.2	2.4	3.6	4.8	6.0	7.2	8.4	9.6	10.8	12.0
Amount of Conjugate Diluent (mL)	12	24	36	48	60	72	84	96	108	120

Working TMB Solution

Note: 1:11 dilution. Bring Chromogen and Substrate Buffer to room temperature. Invert the Chromogen and Substrate Buffer to mix before using. Prepare a 1:11 dilution for each strip to be tested by mixing 100 µL of Chromogen to 1 mL of Substrate Buffer in a clean, **polypropylene** container (**do not use a polystyrene container**). Note Chromogen lot number, date and time of preparation, and date and time of expiration (8 hours from preparation) on container. Mix Working Solution gently when combined and again just prior to use. Working TMB Solution should be kept in the dark at room temperature and used within 8 hours.

Chromogen should be colorless to slightly yellow. Any other color indicates that the reagent is contaminated. Do not use this reagent. The Working TMB Solution should be colorless. A distinct blue color indicates that the reagent is contaminated. Discard the Working TMB Solution and prepare fresh reagent in a clean container.

Prepare only the amount of the reagent to be used within 8 hours, ensuring that the volume of diluted reagent will be adequate for the entire run. Extra Chromogen is provided. Use the following table as a guide:

Preparation of Working TMB Solution by Strip

Number of Strips to be used	1	2	3	4	5	6	7	8	9	10	11	12*
Amount of Chromogen (µL)	100	200	300	400	500	600	700	800	900	1000	1100	1200
Amount of Substrate Buffer (mL)	1	2	3	4	5	6	7	8	9	10	11	12

* Complete Plate

Preparation of Working TMB Solution by Plate

Number of Complete Plates to be used	1	2	3	4	5	6	7	8	9	10
Amount of Chromogen (mL)	1.2	2.4	3.6	4.8	6.0	7.2	8.4	9.6	10.8	12.0
Amount of Substrate Buffer (mL)	12	24	36	48	60	72	84	96	108	120

Wash Solution

Prepare Wash Solution by adding one part Wash Solution Concentrate (30X) to 29 parts of water (e.g., 120 mL of Wash Solution Concentrate to 3480 mL of water). Any lot of Wash Solution Concentrate, provided it is catalog number 25261 and within its labeled shelf life, may be used with this assay. Use deionized or distilled water. Clinical laboratory reagent water is acceptable. The diluted Wash Solution can be stored at room temperature for up to four weeks in a plastic container. Note the lot number, date prepared, and expiration date on the container. Prepare a sufficient quantity of Wash Solution to complete a full run.

8 - SPECIMEN COLLECTION, PREPARATION, AND STORAGE

Serum, plasma, or cadaveric serum specimens may be used in the test. The following anticoagulants, including those in both glass and plastic tubes, have all been evaluated and found to be acceptable: EDTA, sodium and lithium heparin, sodium citrate, CPD, CPDA-1, and ACD. Samples that are collected into anticoagulant tubes should be filled as labeling indicates to avoid improper dilution. SST tubes are acceptable for use, both with and without activator. Specimens with observable particulate matter should be clarified by centrifugation prior to testing. No clinically significant effect has been detected in assay results of serum or plasma samples with increased levels of protein, lipids, bilirubin, or hemolysis, or after heat inactivation of patient samples. Cadaveric serum samples with increased levels of hemolysis have been tested, and no clinically significant effect has been detected in assay results. Note: Cadaveric serum samples with increased levels of protein, lipids, bilirubin, or microbiological contaminants have not been available to evaluate with this assay.

Specimens may be stored at 2-8°C for 7 days. For long-term storage, the specimens should be frozen (at -20°C or colder). Samples should not be used if they have incurred more than 5 freeze-thaw cycles. Mix samples thoroughly after thawing.

Note: If specimens are to be shipped, they should be packed in compliance with Federal Regulations covering the transportation of etiologic agents. Studies have demonstrated that specimens may be shipped refrigerated (2-8°C) or at ambient temperature (≤ 37°C) for up to 7 days. For shipments that are in transit for more than 7 days, specimens should be kept frozen (-20°C or lower).

This kit is not licensed for use with specimens other than serum, plasma, or cadaveric serum specimens. This kit is not intended for use on saliva/oral fluid or urine samples.

9 - GS HIV-1/HIV-2 *PLUS O* EIA PROCEDURE

Materials Provided

See REAGENTS section on page 7.

Materials Required But Not Provided

1. Precision pipettes that deliver 20 to 200 µL, 1 mL, 10 mL, 25 mL, and 50 mL, as needed (accurate within ±10%), and corresponding pipette tips; multichannel pipettors capable of delivering 25 µL and 100 µL are optional.
2. Appropriately sized graduated cylinders.
3. Dry-heat incubator capable of maintaining 37 ± 2°C.
4. Microwell plate or strip washer qualified for use with this assay. The washer must be capable of dispensing 400 µL per well, cycling 5 times, and soaking for 30-60 seconds between each wash.
5. Microwell strip reader qualified for use with this assay. The spectrophotometer should have the following specifications at wavelength 450 nm:

   Bandwidth: 10 nm HBW (Half Band Width) or equivalent
   Absorbance Range: 0 to 2 AU (Absorbance Units)
   Repeatability: ± (0.5% + 0.005) AU
   Linearity or Accuracy: 1% from 0 to 2.0 AU

   The instrument should contain a reference filter for reading at 615 to 630 nm.
6. The GS HIV-1/HIV-2 *PLUS O* EIA may be used with the EVOLIS® and Elite™ instrument systems as an aid in the diagnosis of infection with HIV-1 and/or HIV-2. Note: For availability of these systems in your area, contact Bio-Rad Technical Service.
7. The GS HIV-1/HIV-2 *PLUS O* EIA is approved for use with the ORTHO® Summit System (OSS) as a screening test for human serum and plasma specimens. Within the OSS the operator can process by three different modes: manually (manual pipetting + manual processing), semi-automated (automated pipetting + manual processing), or automated

(automated pipetting + automated processing). The automated mode can consist of the stand-alone ORTHO VERSEIA® Pipetter with the stand-alone ORTHO® Summit Processor (OSP), or the ORTHO VERSEIA® Pipetter integrated with the OSP as the ORTHO VERSEIA® Integrated Processor (VIP). The GS HIV-1/HIV-2 *PLUS O* EIA ORTHO® Assay Protocol Disk is available from ORTHO® Clinical Diagnostics.

8. Household bleach (5% to 8% sodium hypochlorite) which may be diluted to a minimum concentration of 10% bleach (or 0.5% sodium hypochlorite). Alternative disinfectants include 70% ethanol or 0.5% Wescodyne™.
9. Paper towels or absorbent pads for blotting.
10. Labeled null strips for testing partial plates.
11. Clean polypropylene container for preparation of Working TMB Solution (do not use polystyrene). Clean container for preparation of Working Conjugate Solution, 15 or 50 mL.
12. Deionized or distilled water. Clinical laboratory reagent water is acceptable.[41]
13. Gloves.
14. Laboratory timer.
15. EIA reagent reservoirs (optional).
16. Plate Sealers (Catalog #0210-00, or equivalent) are required, except on an approved microplate processor.

Preliminary Statements

1. The expected run time for this procedure is approximately 2.5 - 3 hours from initiation of the first incubation step. Each run of this assay must proceed to completion without interruption after it has been started. The maximum allowable time from start of pipetting to start of incubation is ≤ 1 hour.
2. Using the kit controls, three wells of Negative Control, one well of HIV-1 Positive Control, one well of HIV-2 Positive

Control, and one well of HIV-1 Group O Positive Control must be run on each plate. Assay validity and the cutoff for patient samples are determined by the controls on each individual plate.

3. Do not splash controls, specimens, or reagents between microwells of the plate.
4. Cover plates for each incubation step using plate sealers or other appropriate means to minimize evaporation.
5. Avoid exposure of the plates to light during the final incubation step (following the addition of Working TMB Solution).
6. Adhere to the recommended time constraints for the use of the Working TMB Solution (8 hours), Working Conjugate Solution (8 hours), and Working Wash Solution (4 weeks).
7. Avoid the formation of air bubbles in each microwell.
8. Avoid bumping plates containing liquid reagents (especially Working Conjugate Solution) to prevent adherence of liquid to the plate sealer and/or top edges of the microwells.
9. Adequate washing of the microwells with a validated microplate washer is essential to eliminate non-specific binding.
10. Dry residue from the plate blocking process may be visible in the microwells. Assay results will not be affected by this material. Before reading the plates, carefully wipe the bottom of the plates to remove any material that remains on the outside of the wells, and ensure that all strips have been pressed firmly into place.
11. For additional procedural instructions when running this assay with the EVOLIS® Automated Microplate System, consult the following documents:

    - EVOLIS® MATRM, Assay Module GS HIV-1/HIV-2 *PLUS O* EIA
    - EVOLIS® Operator's Manual

12. For additional procedural instructions when running this assay with the Elite™ Automated Microplate System, consult the Elite™ User Manual or the Elite™ Microplate Assay Testing Reference Manual for this assay.
13. For additional procedural instructions when running this assay with the ORTHO® Summit System, refer to the following documents:
    - ORTHO® Summit System User's Guide
    - ORTHO® Summit Processor User's Guide
    - ORTHO VERSEIA® User's Guide
    - ORTHO VERSEIA® Integrated Processor User's Guide
14. Prior to opening the plate pouch for the first time, verify that some dark blue/purple granules remain inside the dessicant pouch. A completely pink dessicant indicates this plate pouch should not be used.

EIA Procedure

1. Perform equipment maintenance and calibration, where necessary, as required by the manufacturer.
2. **Bring all of the reagents except Conjugate Concentrate to room temperature** before beginning the assay procedure.
3. Prepare Working Conjugate Solution, Working TMB Solution, and Working Wash Solution if not previously prepared. Mix gently by inversion. Mix again just before use.
4. Remove strips not needed for the assay and replace them with labeled null strips, if necessary. Take care when assembling partial plates with coated and uncoated (null) strips, as automated systems cannot distinguish between the strips and will report results for all wells that are assigned a sample ID number (even if a null strip is inadvertently placed where sample IDs have been assigned).

5. Microwell strips not needed for the assay may be returned to the plate pouch and sealed, and then used at a later time. Strips from different plates can only be mixed to assemble full or partial plates if they are from the same plate lot and have come from plates that have previously been tested with kit controls and yielded valid runs. When assembling a plate that contains strips from a newly opened, previously untested plate, one of these strips should be placed at the beginning of the plate and tested with the kit controls.
6. If sample identity is not maintained by an automatic procedure, identify the individual wells for each specimen or control on a data sheet.
7. **Dilute specimens and controls 3:4 in the Specimen Diluent.** Specimens and controls may be prediluted 3:4 in the Specimen Diluent prior to addition of the diluted specimen or control to the well (for example, dilute 150 µL of specimen in 50 µL of Specimen Diluent and then transfer 100 µL to the well), or diluted in-well (add 25 µL of Specimen Diluent to each well first, followed by 75 µL of specimen or controls). Three Negative Controls, one HIV-1 Positive Control, one HIV-2 Positive Control, and one HIV-1 Group O Positive Control must be assayed on each plate or partial plate of specimens. Mix each diluted specimen and control thoroughly. Mix gently to avoid foaming of the diluent. All microwell plates containing controls and specimens must be subjected to the same process and incubation times.
NOTE: After adding the sample, the diluent will change from purple to a blue color.
8. **Add 100 µL of diluted specimen or control to the appropriate wells** of the microwell plate.
9. Cover the microwell plate with a plate sealer or use other means to minimize evaporation. **Incubate the plate for 60 ± 5 minutes at 37 ± 2°C.**
10. At the end of the incubation period, carefully remove the plate cover and aspirate the fluid from each well into a

biohazard container. **Wash the microwell plate or strip a minimum of five times** with the Wash Solution (at least 400 µL/well/wash). Soak each well for 30 to 60 seconds between each wash cycle. Aspirate the Wash Solution after each wash. After the last wash, if excess liquid remains, blot the inverted plate on clean, absorbent paper towels. NOTE: Grasp the plate holder firmly at the center of the long sides before inverting to blot.

11. **Add 100 µL of Working Conjugate Solution (green solution) to each well** containing a specimen or control. NOTE: Avoid bumping plates containing Working Conjugate Solution to prevent contamination of the plate sealer and/or top edges of the wells.
12. Cover the microwell plate with a plate sealer or use other means to minimize evaporation. **Incubate the plate for 30 ± 5 minutes at 37 ± 2°C.**
13. At the end of the incubation period, carefully remove the plate cover and aspirate the fluid in each well into a biohazard container. **Wash the plate a minimum of five times** with Wash Solution (at least 400 µL/well/wash). Soak each well for 30 to 60 seconds between each wash cycle. Aspirate the Wash Solution after each wash. After the last wash, if excess liquid remains, blot the inverted plate on a clean, absorbent paper towel. NOTE: Grasp the plate holder firmly at the center of the long sides before inverting to blot.
14. **Add 100 µL of the Working TMB Solution to each well containing a specimen or control. Incubate the plates in the dark for 30 ± 5 minutes at room temperature (18 to 30°C). Use of a plate sealer or cover is optional.**
15. Carefully remove the plate cover, if used, and **add 100 µL of Stopping Solution to each well** to terminate the reaction. **Tap the plate gently, or use other means to assure complete mixing. Complete mixing is required for acceptable results.**

16. **Read absorbance within 30 minutes** after adding the Stopping Solution, using the 450 nm filter with 615 to 630 nm as reference. Ensure that all strips have been pressed firmly into place before reading.

Decontamination

Dispose of all specimens and materials used to perform the test as though they contain an infectious agent. Disposal should comply with all applicable waste disposal requirements.

10-QUALITY CONTROL - VALIDATION OF RESULTS

Mean Negative Control absorbance value (NCx)

Determine the mean absorbance for the Negative Control by dividing the sum of the absorbance values by the number of acceptable controls. The individual absorbance values of the Negative Control must be greater than 0.000 AU and less than or equal to 0.150 AU. One Negative Control absorbance value may be discarded if it is outside this range. The NCx may be calculated from the two remaining absorbance values.

Negative Control Sample Number	Absorbance	Total Absorbance	=	0.239 =	0.080 (NCx)
1	0.075	3		3	
2	0.083				
3	0.081				
	0.239				

Calculation of Results:

Cutoff Value

Determine the cutoff value by adding the NCx to 0.250 as shown in the example below:

NCx = 0.080
Cutoff Value = 0.080 + 0.250 = 0.330

Assay Validation

A run is valid if the following criteria are met:

- The absorbance values of the individual Negative Controls are greater than 0.000 AU and less than or equal to 0.150 AU. One Negative Control value may be discarded. If two or more Negative Controls are out of limit, the assay must be repeated.
- The absorbance value of the HIV-1 Positive Control must be greater than or equal to 0.700 AU.
- The absorbance value of the HIV-2 Positive Control must be greater than or equal to 0.700 AU.
- The absorbance value of the HIV-1 Group O Positive Control must be greater than or equal to 0.700 AU.

If any of these criteria have not been met, the assay is invalid and must be repeated.

11-INTERPRETATION OF RESULTS

The presence or absence of HIV Ab is determined by relating the absorbance value of the specimen to the cutoff value.

Specimens with absorbance values that are < 0.000 must be repeated. Those with values greater than the upper linearity limits of the reader should be reported as reactive.

Specimens with absorbance values less than the cutoff value are considered non-reactive by the GS HIV-1/HIV-2 *PLUS O* EIA and may be considered negative for HIV-1 (M and O Groups) and HIV-2 antibodies. Further testing is not required.

Specimens with absorbance values greater than or equal to the cutoff value are considered initially reactive by the GS HIV-1/HIV-2 *PLUS O* EIA. Initially reactive specimens should be retested in duplicate to validate the initial test results. Repeat testing of intially reactive specimens processed manually or with the ORTHO® Summit System should be retested either manually or with the ORTHO® Summit System. If, after repeat testing, the

absorbance values of both duplicate specimens are less than the cutoff value, the original specimen may be considered non-repeatedly reactive and negative for HIV-1 (Groups M and O) and HIV-2 antibodies.

If, after repeat testing, the absorbance value of either of the duplicates is greater than or equal to the cutoff value, the specimen must be considered repeatedly reactive.

If the specimen is repeatedly reactive, the probability that antibodies to HIV-1 and/or HIV-2 are present is high, especially for specimens obtained from subjects at increased risk for HIV-1 and/or HIV-2 infection, or for specimens with very high absorbance values. In most settings, it is appropriate to investigate repeatedly reactive specimens by additional, more specific or supplemental tests, such as Western blot or immunofluorescence.

- Specimens that are repeatedly reactive by the GS HIV-1/HIV-2 *PLUS O* EIA and are found to be positive for antibodies to HIV-1 by additional, more specific or supplemental testing but negative or indeterminate for antibodies to HIV-2 are considered to be positive for antibodies to HIV-1.
- Specimens that are repeatedly reactive by the GS HIV-1/HIV-2 *PLUS O* EIA and are found to be positive by additional, more specific or supplemental testing for antibodies to HIV-2, but negative or indeterminate for antibodies to HIV-1, are considered to be positive for antibodies to HIV-2.
- Specimens that are repeatedly reactive by the GS HIV-1/HIV-2 *PLUS O* EIA and are found to be positive by additional, more specific or supplemental testing for both HIV-1 and HIV-2 antibodies may contain antibodies that cross-react with both virus types, or may be indicative of a dual infection with both HIV-1 and HIV-2.

- The interpretation of results of specimens found to be repeatedly reactive by GS HIV-1/HIV-2 *PLUS O* EIA and negative or indeterminate on additional, more specific testing for antibodies to both HIV-1 and HIV-2 is unclear. Clarification may sometimes be obtained by testing another specimen taken three to six months later.

12-LIMITATIONS OF THE PROCEDURE

1. The GS HIV-1/HIV-2 *PLUS O* EIA Procedure and the Interpretation of Results must be followed closely when testing for the presence of antibodies to HIV-1 and/or HIV-2 in plasma, serum, or cadaveric serum specimens. The user of this kit is advised to read the package insert carefully prior to conducting the test. In particular, the test procedure must be carefully followed for sample and reagent pipetting, plate washing, and time and temperature of the incubation steps. Testing of other body specimens, pooled blood or processed plasma, and products made from such pools is not recommended.
2. The GS HIV-1/HIV-2 *PLUS O* EIA detects circulating antibodies to HIV-1 (Groups M and O) and HIV-2 and thus is useful in screening blood and plasma donated for transfusion and further manufacture, in screening cadaveric serum for tissue donation, in evaluating patients with signs or symptoms of AIDS, and in establishing prior infection with HIV-1 or HIV-2. Clinical studies continue to clarify and refine the interpretation and medical significance of the presence of antibodies to HIV-1 or HIV-2.[42] Repeatedly reactive specimens must be investigated by additional, more specific, or supplemental tests. Recommendations for appropriate use of such additional tests may be issued periodically by the United States Public Health Service. For individuals who are confirmed positive for antibodies, appropriate counseling and medical evaluation should be offered. Both confirmation of the test result on a freshly

drawn sample and counseling should be considered an important part of testing for antibody to HIV-1 and HIV-2.

3. AIDS and AIDS-related conditions are clinical syndromes and their diagnosis can only be established clinically.[42] Testing alone cannot be used to diagnose AIDS, even if the recommended investigation of reactive specimens suggests a high probability that the antibody to HIV-1 or HIV-2 is present.
4. A negative test result at any point in the investigation of individual subjects does not preclude the possibility of exposure to or infection with HIV-1 and/or HIV-2.
5. Negative results can occur if the quantity of marker present in the sample is too low for the detection limits of the assay, or if the marker which is detected is not present during the stage of disease in which a sample is collected.
6. Failure to add specimen or reagent as instructed in the procedure could result in a falsely negative test. Repeat testing should be considered where there is clinical suspicion of infection or procedural error.
7. The risk of an asymptomatic person with a repeatedly reactive test developing AIDS or an AIDS-related condition is not known, as the course of HIV infection may vary among individual patients and may be altered by antiretroviral therapy.[43,44] However, in a prospective study, AIDS developed in 51% of homosexual men after 10 years of infection.[45]
8. Data obtained from testing persons both at increased and at low risk for HIV-1 and/or HIV-2 infection suggest that repeatedly reactive specimens with high reactivity on the GS HIV-1/HIV-2 *PLUS O* EIA may be more likely to demonstrate the presence of antibodies to HIV-1 (Groups M and O) and/or HIV-2 by additional, more specific, or supplemental testing.[46] Borderline reactivity is more frequently nonspecific, especially in samples obtained from persons at low risk for infection with HIV-1 or HIV-2; however, the presence of

antibodies to HIV-1 and/or HIV-2 in some of these specimens can be demonstrated by additional, more specific, or supplemental testing, or by testing a subsequent sample drawn at a later date (e.g. 3 to 6 months).[47]

9. It is generally recognized that detection of HIV antibody in infants born to seropositive mothers is not adequate to diagnose HIV infection in the infant, since maternal IgG frequently persists for as long as 18 months after birth. Supplemental assays designed specifically for neonatal specimens may be helpful in resolving such cases.[48]
10. An absorbance value of less than 0.000 AU may indicate a procedural or instrument error that should be evaluated. That result is invalid and that specimen must be re-run.
11. Factors that can affect the validity of results include failure to add the specimen or reagents to the well, inadequate washing of microplate wells, failure to follow stated incubation times and temperatures, addition of wrong reagents to wells, the presence of metals, or splashing of bleach into wells.
12. Non-repeatedly reactive specimens can be caused by:
    - improper washing of microwell plates during the initial test
    - cross-contamination of nonreactive specimens with HIV antibody from a high-titered specimen
    - contamination of the Chromogen or Working TMB Solution by oxidizing agents (sodium hypochlorite, hydrogen peroxide, etc.)
    - contamination of the Stopping Solution
13. A person who has antibodies to HIV-1 is presumed to be infected with the virus, except that a person who has participated in an HIV vaccine study may develop antibodies to the vaccine and may or may not be infected with HIV. Clinical correlation is indicated with appropriate counseling,

medical evaluation, and possibly additional testing to decide whether a diagnosis of HIV infection is accurate.

13-PERFORMANCE CHARACTERISTICS OF SERUM AND PLASMA TESTING

Reproducibility—Manual Testing

A panel of 14 specimens was used for determining the reproducibility of the GS HIV-1/HIV-2 *PLUS O* EIA. The 14 member panel included 5 dilutions of an HIV-1 antibody positive sample (Group M); one HIV-1 (Group O) antibody positive sample (rabbit); 6 dilutions of an HIV-2 antibody positive sample; and two undiluted HIV-negative samples.

The composition of the panel was as follows:

#	Panel Member Composition	#	Panel Member Composition
1	HIV-1 Positive	8	HIV-2 Positive
2	HIV-1 Positive	9	HIV-2 Positive
3	HIV-1 Group O Positive	10	HIV-2 Low Positive
4	HIV-1 Low Positive	11	HIV-2 High Negative
5	HIV-1 High Negative	12	HIV-2 Negative
6	HIV Negative	13	HIV Negative
7	HIV-2 Positive	14	HIV Negative

The specimens were tested in triplicate on 3 different days using 3 different test kit lots at each of 6 sites. The data were analyzed according to the principles described in the Clinical and Laboratory Standards Institute guidance (CLSI) EP15-A2. The standard deviation (SD) and percent coefficient of variation (%CV) were calculated from the observed Optical Density (OD) and Signal to Cutoff values, respectively, for each panel member and are presented in the tables below.

Table 1a: Reproducibility of the GS HIV-1/HIV-2 *PLUS O* EIA by Optical Density (OD) values

Panel Member	N[a]	Mean (OD)	Within Run		Between Day		Between Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	162	1.240	0.113	9.1	0.039	3.2	0.000	0.0	0.120	9.7
2	162	0.972	0.081	8.3	0.047	4.8	0.052	5.3	0.107	11.0
3	162	0.485	0.116	23.9	0.000	0.0	0.080	16.5	0.141	29.0
4	161	0.423	0.042	10.0	0.019	4.4	0.013	3.1	0.048	11.4
5	162	0.255	0.028	11.1	0.016	6.1	0.021	8.2	0.039	15.1
6	161	0.128	0.021	16.8	0.008	6.3	0.008	6.0	0.024	18.9
7	162	1.195	0.132	11.1	0.066	5.5	0.130	10.9	0.197	16.5
8	162	0.845	0.103	12.2	0.037	4.4	0.113	13.3	0.157	18.6
9	162	0.648	0.089	13.7	0.031	4.8	0.068	10.5	0.116	17.9
10	162	0.401	0.042	10.4	0.026	6.5	0.052	13.1	0.072	18.0
11	162	0.236	0.024	10.1	0.016	6.7	0.028	12.0	0.040	17.0
12	162	0.112	0.012	10.3	0.007	6.4	0.011	9.7	0.017	15.5
13	162	0.041	0.006	14.4	0.004	8.9	0.008	19.0	0.010	25.4
14	162	0.044	0.009	21.5	0.002	5.4	0.008	17.4	0.012	28.2

a. Outliers not included in statistical calculations.

Table 1b: Reproducibility of the GS HIV-1/HIV-2 *PLUS O* EIA by Signal to Cutoff (S/CO) values

Panel Member	N[a]	Mean (S/CO)	Within Run		Between Day		Between Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	162	4.09	0.370	9.0	0.125	3.1	0.035	0.9	0.392	9.6
2	162	3.21	0.254	7.9	0.139	4.3	0.220	6.9	0.364	11.3
3	162	1.60	0.373	23.3	0.000	0.0	0.280	17.5	0.466	29.1
4	161	1.40	0.141	10.1	0.048	3.5	0.048	3.4	0.156	11.2
5	162	0.84	0.085	10.2	0.044	5.2	0.072	8.6	0.120	14.3
6	161	0.42	0.069	16.3	0.029	6.9	0.014	3.4	0.076	18.1
7	162	3.94	0.452	11.5	0.163	4.1	0.422	10.7	0.640	16.3
8	162	2.79	0.362	13.0	0.077	2.8	0.362	13.0	0.518	18.6
9	162	2.14	0.292	13.7	0.087	4.1	0.232	10.8	0.383	17.9
10	162	1.32	0.145	11.0	0.070	5.3	0.169	12.8	0.233	17.7
11	162	0.78	0.083	10.7	0.043	5.6	0.092	11.8	0.131	16.9
12	162	0.37	0.037	10.1	0.018	5.0	0.032	8.7	0.053	14.3
13	162	0.14	0.018	13.0	0.011	8.0	0.024	17.7	0.032	23.4
14	162	0.14	0.029	20.1	0.007	4.7	0.023	15.9	0.037	26.0

a. Outliers not included in statistical calculations.

Reproducibility and Precision Testing—EVOLIS® Automated Microplate System

A panel of 15 specimens was used for determining the reproducibility and precision of the GS HIV-1/HIV-2 *PLUS O* EIA. The 15 member panel included 6 serum members (5 positive and 1 negative), 5 plasma members (4 positive and 1 negative), and the 4 GS HIV-1/HIV-2 *PLUS O* EIA kit controls. The composition of the panel was as follows:

#	Panel Member Composition
1	HIV-1 Moderate Positive (Serum)
2	HIV-1 Moderate Positive (Plasma)
3	HIV-1 Low Positive (Serum)
4	HIV-1 Low Positive (Plasma)
5	HIV-2 Moderate Positive (Serum)
6	HIV-2 Moderate Positive (Plasma)
7	HIV-2 Low Positive (Serum)
8	HIV-2 Low Positive (Plasma)
9	HIV-1 Group O Positive (Serum)
10	HIV High Negative (Serum)
11	HIV High Negative (Plasma)
12	HIV-1 Positive Control
13	HIV-2 Positive Control
14	HIV-1 Group O Positive Control
15	HIV Negative Control

Reproducibility

Reproducibility of the GS HIV-1/HIV-2 *PLUS O* EIA was determined for the EVOLIS® Automated Microplate System by testing the panel at three (3) sites on three (3) different EVOLIS® instruments (one at each site) using a single lot of the GS HIV-1/HIV-2 *PLUS O* EIA. Each panel member was tested in triplicate (x3) on 2 runs per day for 5 days (30 replicates per member per lot/site). The data were analyzed according to the principles described in the Clinical and Laboratory Standards Institute guidance (CLSI) EP15-A2. The standard deviation (SD) and percent coefficient of variation (%CV) were calculated for each panel member and are presented in the table below.

Table 2: Reproducibility Results - EVOLIS® Testing By Signal to Cutoff Ratio (S/CO) N = 90 for each panel member[a]

Panel Member	Mean (S/CO)	Within Run		Between Run		Between Day		Between Site		Total[b]	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	2.74	0.114	4.2	0.160	5.8	0.110	4.0	0.116	4.2	0.254	9.3
2	2.34	0.064	2.8	0.120	5.1	0.059	2.5	0.031	1.3	0.151	6.5
3	1.49	0.063	4.2	0.049	3.3	0.045	3.0	0.049	3.3	0.104	7.0
4	1.36	0.055	4.1	0.065	4.8	0.044	3.3	0.068	5.0	0.118	8.7
5	2.19	0.102	4.7	0.299	13.6	0.232	10.6	0.221	10.1	0.450	20.5
6	2.34	0.247	10.6	0.358	15.3	0.057	2.4	0.384	16.4	0.583	24.9
7	1.64	0.056	3.4	0.242	14.8	0.135	8.2	0.177	10.8	0.334	20.4
8	1.67	0.097	5.8	0.309	18.5	0.105	6.3	0.277	16.6	0.439	26.3
9	2.12	0.105	5.0	0.246	11.6	0.166	7.8	0.149	7.0	0.348	16.4
10	0.71	0.028	3.9	0.040	5.6	0.000	0.0	0.012	1.7	0.050	7.0
11	0.75	0.036	4.9	0.032	4.4	0.015	2.0	0.029	3.9	0.058	7.8
12	7.10	0.187	2.6	0.434	6.1	0.164	2.3	0.353	5.0	0.612	8.6
13	6.13	0.186	3.0	0.746	12.2	0.617	10.1	0.462	7.5	1.089	17.8
14	6.49	0.233	3.6	0.419	6.5	0.284	4.4	0.484	7.5	0.738	11.4
15	0.21	0.023	11.1	0.041	19.9	0.000	0.0	0.024	11.5	0.053	25.5

a One (1) result was missing for panel member 2 due to insufficient sample volume.
b Total: Total variability of the assay performance includes within run, between run, between day, and between site.

Precision

Precision of the GS HIV-1/HIV-2 *PLUS O* EIA was determined for the EVOLIS® Automated Microplate System by testing the panel at one (1) site on one (1) EVOLIS® instrument using a single lot of the GS HIV-1/HIV-2 *PLUS O* EIA. Each panel member was tested in duplicate (x2) on two runs per day for a period of 20 days (80 replicates per member). The data were analyzed at Bio-Rad Laboratories according to the principles described in the Clinical and Laboratory Standards Institute guidance (CLSI) EP5-A2. The standard deviation (SD) and percent coefficient of variation (%CV) were calculated for each panel member and are presented in the table below.

Table 3: Precision Results - EVOLIS® Testing By Signal to Cutoff Ratio (S/CO) N = 80

Panel Member	Mean (S/CO)	Within Run		Between Run		Between Day		Total[a]	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	2.84	0.071	2.5	0.090	3.2	0.065	2.3	0.131	4.6
2	2.34	0.062	2.6	0.075	3.2	0.052	2.2	0.110	4.7
3	1.49	0.043	2.9	0.062	4.2	0.036	2.5	0.084	5.7
4	1.49	0.063	4.2	0.048	3.2	0.058	3.9	0.098	6.6
5	1.97	0.065	3.3	0.214	10.9	0.181	9.2	0.288	14.6
6	1.85	0.218	11.8	0.258	14.0	0.161	8.7	0.374	20.3
7	1.51	0.054	3.6	0.177	11.7	0.000	0.0	0.185	12.3
8	1.38	0.052	3.8	0.184	13.3	0.000	0.0	0.191	13.8
9	1.93	0.061	3.2	0.232	12.0	0.000	0.0	0.240	12.4
10	0.75	0.026	3.5	0.031	4.1	0.027	3.6	0.049	6.5
11	0.75	0.026	3.5	0.032	4.3	0.010	1.4	0.043	5.7
12	7.73	0.202	2.6	0.234	3.0	0.331	4.3	0.453	5.9
13	5.87	0.192	3.3	0.428	7.3	0.000	0.0	0.469	8.0
14	6.32	0.155	2.4	0.444	7.0	0.000	0.0	0.470	7.4
15	0.24	0.029	12.2	0.046	19.4	0.000	0.0	0.054	22.9

a Total: Total variability of the assay performance includes within run, between run, and between day.

Reproducibility and Precision Testing - Elite™ Automated Microplate System

The reproducibility and precision of the Elite™ Automated Microplate System with the GS HIV-1/HIV-2 *PLUS O* EIA were determined using a panel of 17 members that consisted of 7 serum, 6 plasma, and 4 kit controls. The composition of the panel is as follows:

#	Panel Member Composition
1	HIV-1 Moderate Positive Plasma
2	HIV-1 Low Positive Plasma
3	HIV-2 Moderate Positive Plasma
4	HIV-2 Low Positive Plasma
5	Low Negative Plasma
6	HIV-1 High Negative Plasma
7	HIV-1 Moderate Positive Serum
8	HIV-1 Low Positive Serum
9	HIV-2 Moderate Positive Serum
10	HIV-2 Low Positive Serum
11	HIV-1 Group O Moderate Positive Serum
12	Low Negative Serum
13	HIV-1 High Negative Serum
14	HIV-1 High Positive Control
15	HIV-2 High Positive Control
16	HIV-1 Group O High Positive Control
17	Low Negative Control

Reproducibility

Reproducibility testing of the GS HIV-1/HIV-2 *PLUS O* EIA was performed on the Elite™ Automated Microplate System at 3 sites using 3 different Elite™ instruments (one at each site), and 1 lot of the GS HIV 1/HIV-2 *PLUS O* EIA. Each panel member was tested in triplicate on 2 runs per day for 5 days (30 replicates per member per site). The data were analyzed according to the principles described in the CLSI guidance EP15-A2. The SD and %CV that were calculated for each panel member are shown in the table below.

Table 4: Reproducibility Results - Elite™ Testing by Signal to Cutoff Ratio (SCO)

Panel Member	#	Mean S/CO	Within Run		Between Run		Between Day		Between Site		Total[a]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	90	2.56	0.130	5.1	0.086	3.4	0.023	0.9	0.150	5.9	0.218	8.5
2	90	1.63	0.051	3.1	0.051	3.1	0.044	2.7	0.093	5.7	0.126	7.7
3	90	2.11	0.233	11.0	0.141	6.7	0.026	1.2	0.146	6.9	0.309	14.7
4	90	1.61	0.199	12.4	0.114	7.1	0.000	0.0	0.166	10.3	0.283	17.6
5	90	0.13	0.019	14.8	0.000	0.0	0.015	11.9	0.021	16.5	0.032	25.2
6	90	0.96	0.052	5.4	0.029	3.1	0.016	1.6	0.051	5.4	0.080	8.4
7	88	2.52	0.058	2.3	0.107	4.2	0.029	1.2	0.139	5.5	0.187	7.4
8	90	1.55	0.051	3.3	0.050	3.2	0.042	2.7	0.113	7.3	0.140	9.0
9	88	2.37	0.158	6.7	0.241	10.2	0.000	0.0	0.093	3.9	0.302	12.8
10	87	1.64	0.117	7.2	0.203	12.4	0.077	4.7	0.134	8.2	0.281	17.2
11	89	1.97	0.144	7.3	0.125	6.4	0.074	3.8	0.000	0.0	0.205	10.4
12	90	0.11	0.012	11.5	0.000	0.0	0.014	13.5	0.033	31.2	0.038	35.9
13	89	0.75	0.026	3.4	0.030	4.0	0.022	2.9	0.022	2.9	0.050	6.7
14	90	6.44	0.225	3.5	0.238	3.7	0.000	0.0	0.337	5.2	0.471	7.3
15	90	5.60	0.308	5.5	0.467	8.3	0.000	0.0	0.190	3.4	0.591	10.6
16	90	4.96	0.209	4.2	0.259	5.2	0.181	3.6	0.000	0.0	0.379	7.7
17	89	0.12	0.023	18.9	0.000	0.0	0.014	11.3	0.008	6.3	0.028	22.9

a. Total: Total variability of the assay performance includes within run, between run, between day, and between site.

Precision

Precision testing of the GS HIV-1/HIV-2 *PLUS O* EIA with the Elite™ Automated Microplate System was performed with one instrument at one site. The 17-member panel was tested in duplicate on two runs per day for 12 days on 1 lot of the GS HIV-1/HIV-2 *PLUS O* EIA assay (total number of replicates per each assay = 2 (in duplicate) x 2 runs/day x 12 days = 48

replicates per panel member). The data were analyzed according to the principles described in the CLSI guidance EP5-A2. The SD and %CV that were calculated for each panel member are shown in the table below.

Table 5: Precision Results - Elite™ Testing by Signal to Cutoff Ratio (SCO)

Panel Member	N	Mean S/CO	Within Run		Between Run		Between Day		Total[a]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	48	2.87	0.124	4.3	0.053	1.9	0.094	3.3	0.164	5.7
2	48	1.77	0.041	2.3	0.055	3.1	0.020	1.1	0.072	4.1
3	48	2.03	0.128	6.3	0.126	6.2	0.095	4.7	0.203	10.0
4	48	1.42	0.104	7.3	0.140	9.9	0.000	0.0	0.175	12.3
5	48	0.12	0.013	10.7	0.005	4.4	0.022	18.6	0.026	21.9
6	48	1.00	0.040	4.0	0.025	2.5	0.023	2.3	0.052	5.2
7	47*	2.66	0.059	2.2	0.038	1.4	0.064	2.4	0.094	3.6
8	47*	1.68	0.034	2.0	0.034	2.0	0.048	2.9	0.068	4.0
9	48	2.37	0.150	6.4	0.205	8.7	0.000	0.0	0.254	10.8
10	48	1.65	0.136	8.2	0.166	10.0	0.000	0.0	0.214	13.0
11	47*	1.87	0.083	4.4	0.177	9.5	0.136	7.3	0.238	12.8
12	48	0.12	0.019	16.3	0.000	0.0	0.032	27.3	0.037	31.8
13	47*	0.85	0.022	2.6	0.026	3.1	0.067	7.9	0.075	8.9
14	48	6.90	0.166	2.4	0.189	2.7	0.062	0.9	0.259	3.8
15	48	5.91	0.258	4.4	0.595	10.1	0.079	1.3	0.653	11.0
16	48	5.12	0.115	2.2	0.240	4.7	0.309	6.0	0.408	8.0
17	48	0.16	0.020	12.8	0.000	0.0	0.036	23.4	0.041	26.6

a. Total: Total variability of the assay performance includes within run, between run, between site, and between days.

SENSITIVITY AND SPECIFICITY

Testing to determine the sensitivity and specificity of the GS HIV-1/HIV-2 *PLUS O* EIA was performed manually, and specificity was also determined with the ORTHO® Summit System (using both the ORTHO® Summit Sample Handling System* and the ORTHO VERSEIA® Pipetter). In addition, comparative sensitivity and specificity studies were used to evaluate use of the EVOLIS® and Elite™ Automated Microplate Systems, and comparative sensitivity studies were used to evaluate the ORTHO® Summit System. Unless otherwise noted in the tables that follow, the results summarize the manual testing that was performed. The results of the testing with the EVOLIS®, Elite™, and ORTHO® Summit Systems are described in text, where applicable.

Specificity Studies

Reactivity in Random Blood Donors and Individuals with Medical Conditions Unrelated to HIV-1 or HIV-2

The results of testing specimens from random blood and plasma donors and specimens from individuals with medical conditions unrelated to HIV-1 or HIV-2 infection with the GS HIV-1/HIV-2 *PLUS O* EIA are summarized in Tables 6 and 7. The data include serum and plasma samples obtained from donors at 3 geographically distinct locations, and 360 specimens from individuals with various medical conditions.

* The ORTHO® Summit Sample Handling System is now a legacy device and no longer available for marketing.

Table 6: Detection of Antibodies to HIV-1 and/or HIV-2 in Random Donors

Results Obtained with GS HIV-1/HIV-2 *PLUS O* EIA					Repeatedly Reactive Specimens	
Group (Sample Type = Serum)	Number Tested	Non-Reactive	Initially Reactive	Repeatedly Reactive	HIV-2 EIA Repeatedly Reactive	Pos. by HIV-1 Immunoblot alone
Random Blood Donors, Site 1	2999 (100.00%)	2996 (99.90%)	3 (0.10%)	3 (0.10%)	0	0
Random Blood Donors, Site 2	3104 (100.00%)	3101 (99.90%)	3 (0.10%)	3 (0.10%)	0	0
SUB TOTAL:	6103 (100.00%)	6097 (99.90%)	6 (0.10%)	6 (0.10%)	0	0

Results Obtained with GS HIV-1/HIV-2 *PLUS O* EIA					Repeatedly Reactive Specimens	
Group (Sample Type = Plasma)	Number Tested	Non-Reactive	Initially Reactive	Repeatedly Reactive	HIV-2 EIA Repeatedly Reactive	Pos. by HIV-1 Immunoblot alone
Random Blood Donors, Site 2	2901 (100.00%)	2895 (99.79%)	6 (0.21%)	4 (0.14%)	0	0
Random Blood Donors, Site 3	2155 (100.00%)	2149 (99.72%)	6 (0.28%)	2 (0.09%)	0	0
SUB TOTAL:	5056 (100.00%)	5044 (99.76%)	12 (0.24%)	6 (0.12%)	0	0
TOTAL: (Serum and Plasma)	**11,159 (100.00%)**	**11,141 (99.84%)**	**18 (0.16%)**	**12 (0.11%)**	**0**	**0**

As shown in Table 6, 99.84% of the normal donor population (n = 11,159) were initially nonreactive, 0.16% were initially reactive, and 0.11% were repeatedly reactive. Twelve of the 18 initially reactive specimens were repeatedly reactive upon retesting. None of the repeatedly reactive specimens were positive for antibodies to HIV-1 or HIV-2 by Western blot.

Specificity of the GS HIV-1/HIV-2 *PLUS O* EIA was estimated from the results of screening tests in random blood and plasma donors, and determined by the following formula:

$$\frac{\text{(\# normal donor specimens - \# repeatedly reactive specimens)}}{\text{(\# normal donor specimens - repeatedly reactive specimens confirmed positive for antibodies to HIV)}} \times 100$$

Thus, assuming a zero prevalence rate of antibodies to HIV-1 and HIV-2 in this population, the GS HIV-1/HIV-2 *PLUS O* EIA

had an estimated specificity in this study of (11,159 – 12) x 100/11,159 = 99.89% (95% confidence interval: 99.83 – 99.96).

Reactivity of Known Negatives Using the EVOLIS® Automated Microplate System

Comparative studies using the EVOLIS® Automated Microplate System in comparison to manual testing with the GS HIV-1/HIV-2 *PLUS O* EIA were performed with specimens known to be negative for HIV antibody. A total of 100 known negative samples were tested once on each of three different instruments at each of three different sites. Approximately 30-40% of the known negative samples were near the assay cut-off. For the 100 known negative samples combined across sites, 99.7% (299/300) of manual results were nonreactive; 100% (300/300) of the EVOLIS® Automated Microplate System results were nonreactive. The negative % agreement was 100% (299/299) with a 95% confidence interval range of 98.7%-100.0%. These studies demonstrate comparable results for both methods when testing with the GS HIV-1/HIV-2 *PLUS O* EIA.

Reactivity of Known Negatives Using the Elite™ Automated Microplate System

The performance of the Elite™ Automated Microplate System was compared to manual testing of the GS HIV-1/HIV-2 *PLUS O* EIA with specimens known to be negative for HIV antibody. A panel of 100 known negative samples was tested once on each of three different instruments at each of three different sites. Approximately 30-40% of the known negative samples were near the assay cut-off. All of the 100 known negative samples were nonreactive at all 3 sites, with both the Elite™ Automated Microplate System and manual testing. Averaged across all sites, the overall % agreement was 100% (100/100) with a 95% confidence interval range of 96.4%-100.0%. These studies demonstrate comparable results for both methods when testing with the GS HIV-1/HIV-2 *PLUS O* EIA.

Random Blood Donors Tested with ORTHO® Summit System

Additional specificity studies have been performed with the GS HIV-1/HIV-2 *PLUS O* EIA using the ORTHO® Summit System with the ORTHO® Summit Sample Handling System.* In total, 24,250 normal donors (including a combination of serum and plasma specimens) were tested at 3 U.S. blood centers. Three (3) samples that were repeatedly reactive and confirmed positive by HIV-1 Western blot were excluded from the specificity analysis. Of the remaining 24,247 samples tested, 52 were initially reactive (0.21%) and 2 of these specimens were QNS for repeat testing or confirmation. There were 17 repeatedly reactive specimens that were either negative (6), indeterminate (9), or not tested (2) by Western blot. Therefore, the GS HIV-1/HIV-2 *PLUS O* EIA had an estimated specificity in this study of 99.92% (24,228/24,247; 95% confidence interval: 99.88 – 99.96).

Specificity studies have also been performed with unlinked random donor serum and plasma specimens on the ORTHO® Summit System using the ORTHO VERSEIA® Pipetter. The combined testing at 3 testing sites showed a specificity of 100% (3216/3216; 95% confidence interval: 99.88 – 100.0%).

Additional studies have also been performed with unlinked random donor serum and plasma specimens with the stand-alone ORTHO VERSEIA® Pipetter and a stand-alone ORTHO® Summit Processor (OSP) in comparison to the ORTHO VERSEIA® Integrated Processor (VIP). Both the VIP system and the stand-alone system showed a specificity of 100% (2019/2019; 95% confidence interval: 99.82 – 100.0%). The VIP S/CO percent difference increased from the stand-alone VERSEIA®/stand-alone OSP by 4.6%.

* The ORTHO® Summit Sample Handling System is now a legacy device no longer available for marketing.

Table 7: Detection of Antibodies to HIV-1 and/or HIV-2 in Individuals with Other Medical Conditions Unrelated to HIV Infection

Group	Results Obtained with GS HIV-1/HIV-2 *PLUS O* EIA				Repeatedly Reactive Specimens	
	Number Tested	Non-Reactive	Initially Reactive	Repeatedly Reactive	HIV-2 EIA Repeatedly Reactive	Pos. by HIV-1 Immunoblot alone
Immunological diseases[a]	80 (100.00%)	78 (97.50%)	2 (2.50%)	2 (2.50%)	0	0
Acute or chronic viral diseases/ parasitic diseases[b]	200 (100.00%)	199 (99.50%)	1 (0.50%)	1 (0.50%)	0	0
Malignancies[c]	20 (100.00%)	20 (100.00%)	0 (0.00%)	0 (0.00%)	NA	NA
Miscellaneous/ Other[d]	60 (100.00%)	59 (98.33%)	1 (1.67%)	1 (1.67%)	0	0
TOTAL:	360 (100.00%)	356 (98.89%)	4 (1.11%)	4 (1.11%)	0	0

a. 20 SLE (ANA positive); 20 Rheumatoid arthritis (RF positive); 20 IgG Hypergammaglobulinemia; 20 IgM Hypergammaglobulinemia
b. 20 HCV; 20 HBV; 20 HAV; 20 CMV; 20 EBV; 20 HSV; 20 HTLV-I/II; 20 Rubella; 20 Syphilis; 20 Toxoplasmosis
c. 2 Adenocarcinoma; 2 Bladder Cancer; 2 Breast Cancer; 3 Colon Cancer; 1 Endometrial Cancer; 1 Lung Cancer; 1 Melanoma, metastatic; 3 Prostate Cancer; 3 Rectal Cancer; 1 Renal Cell Cancer; 1 Squamous Cell Cancer
d. 20 Non Viral Cirrhosis [(primary biliary (5); alcohol induced (8); drug induced (7)]; 20 Multiple Transfusions; 20 Multiparous females

Four specimens from individuals with unrelated medical conditions were initially and repeatedly reactive in the GS HIV-1/HIV-2 *PLUS O* EIA. Of the 4 specimens, 1 was from an individual with cirrhosis; 1 was from an individual with HCV; and 2 were from individuals positive for Rheumatoid Factor (RF). Three of the 4 specimens were negative and 1 was indeterminate when tested with a licensed HIV-1 Western Blot. All of the specimens were nonreactive for antibody to HIV-2 when tested with a licensed HIV-2 EIA. None of the remaining specimens from individuals with other medical conditions were reactive in the GS HIV-1/HIV-2 *PLUS O* EIA. There appears to be no correlation between reactivity in the GS HIV-1/HIV-2 *PLUS O* EIA and other medical conditions unrelated to HIV infection.

Sensitivity Studies

Reactivity in Specimens Known to be Positive for Antibodies to HIV-1

The reactivity of the GS HIV-1/HIV-2 *PLUS O* EIA was determined by testing serum and plasma samples from patients diagnosed as having AIDS (n = 313), and from 689 individuals known to be HIV-1 antibody positive from U.S. (n = 490) and non-U.S. locations (n = 199[a]) for whom the clinical status was unknown. The results of testing are shown in Table 8.

Table 8: Reactivity in HIV-1 Known Positive Specimens

Group	GS HIV-1/HIV-2 *PLUS O* EIA No. Repeatedly Reactive	Licensed HIV-1/HIV-2 EIA No. Repeatedly Reactive
AIDS (N = 313)	313 (100%)	313 (100%)
Known HIV-1 Positive U.S. (N = 490)	490 (100%)	490 (100%)
Known HIV-1 Positive non U.S. (N = 199[a])	199 (100%)	199 (100%)
TOTAL	1002 (100%)	1002 (100%)

a. Australia, New S. Wales (N = 36)
Central African Republic (N = 40)
Ghana (N = 5)
Kenya (N = 3)
Nigeria (N = 46)
Sierra Leone (N = 40)
Thailand (N = 21)
Zimbabwe (N = 8)

Of the 313 diagnosed AIDS patients, 100% were repeatedly reactive with the GS HIV-1/HIV-2 *PLUS O* EIA. All AIDS specimens were positive on a licensed HIV-1 Western blot. Of the known 689 positives from U.S. and non-U.S. locations, all were confirmed positive with one of four licensed HIV-1 Western blots.

The HIV-1 sensitivity of the GS HIV-1/HIV-2 *PLUS O* EIA was estimated from the results of testing 313 patients with AIDS. A positive test result was obtained for 313 of 313 patients for an estimated sensitivity in this study of 100% (95% confidence interval: 99.84% to 100%).

Reactivity in Specimens from High-Risk Individuals from the United States and Canada

A total of 1011 specimens from high-risk individuals from public health labs in the United States and Canada were tested with the GS HIV-1/HIV-2 *PLUS O* EIA. Results of testing individuals from the United States (n = 761) and Canada (n = 250) are shown in Table 9. All specimens were screened with one or more FDA and/or Canadian licensed HIV-1/HIV-2 EIAs. All specimens repeatedly reactive with the GS HIV-1/HIV-2 *PLUS O* EIA and/or the licensed HIV-1/HIV-2 EIAs were tested with a licensed HIV-1 Western blot. If a specimen tested negative or indeterminate on the licensed HIV-1 Western blot, it was tested with a licensed HIV-2 EIA. If a specimen was repeatedly reactive on the licensed HIV-2 EIA, and negative or indeterminate on the HIV-1 Western blot, it was additionally tested with an in-house HIV-2 Western blot.

Table 9: Reactivity in Specimens from High-Risk Individuals from the United States and Canada

Group	No. Tested	GS HIV-1/HIV-2 *PLUS O* EIA Repeatedly Reactive	No. RR on one or more licensed HIV-1/HIV-2 EIA	No. Pos. by HIV-1 Western blot
U.S	761	36 (4.7%)[a]	22 (2.9%)[a]	17 (2.3%) [a]
Canada	250	3 (1.2%)[b]	2 (0.8%)[b]	2 (0.8%) [b]
Total	1011	39 (3.9%)	24 (2.4%)	19 (1.9%)

a. Seventeen (17) specimens were repeatedly reactive on both the GS HIV-1/HIV-2 *PLUS O* EIA and one or more licensed HIV-1/HIV-2 EIAs.
b. Two (2) specimens were repeatedly reactive on both the GS HIV-1/HIV-2 *PLUS O* EIA and one or more licensed HIV-1/HIV-2 EIAs.

Twenty-five specimens were additionally tested with a licensed HIV-2 EIA (20 specimens were repeatedly reactive on the GS HIV-1/HIV-2 *PLUS O* EIA only, and 5 specimens were repeatedly reactive on the licensed HIV-1/HIV-2 EIA.) All 25 specimens were negative or indeterminate on a licensed HIV-1 Western blot. Of the 25 specimens tested with a licensed HIV-2 EIA, none were repeatedly reactive.

Therefore, the GS HIV-1/HIV-2 *PLUS O* EIA detected all HIV-1 confirmed positives [19/19 (100%)] in this study of high-risk populations in the United States and Canada.

Reactivity in Prospectively Obtained Public Health Specimens

The GS HIV-1/HIV-2 *PLUS O* EIA was evaluated in prospective public health populations. The samples collected and tested at two public health labs excluded individuals reporting high-risk behaviors, whereas the third lab did not exclude such high-risk individuals. The data include 1501 serum specimens tested at two U.S. and one Canadian location. All specimens were tested with the GS HIV-1/HIV-2 *PLUS O* EIA and an FDA licensed HIV-1/HIV-2 EIA. Specimens repeatedly reactive with the GS HIV-1/HIV-2 *PLUS O* EIA and/or the licensed HIV-1/HIV-2 EIA were tested with a licensed HIV-1 Western blot. Specimens that were repeatedly reactive with the GS HIV-1/HIV-2 *PLUS O* EIA and/or the licensed HIV-1/HIV-2 EIAs were tested with a licensed HIV-2 EIA if the HIV-1 Western blot was negative or indeterminate. If a specimen was repeatedly reactive on the licensed HIV-2 EIA, and negative or indeterminate on the HIV-1 Western blot, it was tested with an in-house HIV-2 Western blot.

Table 10: Reactivity with GS HIV-1/HIV-2 *PLUS O* EIA Prospective Public Health Specimens

Results Obtained with GS HIV-1/HIV-2 *PLUS O* EIA					Licensed EIA Repeatedly Reactive	Repeatedly Reactive Specimens	
Group	Number Tested	Non-Reactive	Initially Reactive	Repeatedly Reactive		HIV-2 EIA Repeatedly Reactive	Pos. by HIV-1 Immunoblot alone
Site 1	501 (100.00%)	495 (98.80%)	6 (1.20%)	2 (0.40%)	1 (0.20%)	1*	0
Site 2	500 (100.00%)	481 (96.20%)	19 (3.80%)	18 (3.60%)	17 (3.40%)	0	13**
Site 3	500 (100.00%)	498 (99.60%)	2 (0.40%)	2 (0.40%)	3 (0.60%)	0	1**
SUBTOTAL:	1501 (100.00%)	1474 (98.20%)	27 (1.80%)	22 (1.47%)	21 (1.40%)	1	14**
(Minus HIV-1 Immunoblot Positive) TOTAL:	1501 - 14 1487 (100.00%)	1474 (99.13%)	27 -14 13 (0.87%)	22 -14 8 (0.54%)	21 -14 7 (0.47%)		

*This specimen, which was repeatedly reactive only on the GS HIV-1/HIV-2 *PLUS O* EIA, was indeterminate when tested on an in-house HIV-2 Western Blot.
**These specimens were repeatedly reactive on both the GS HIV-1/HIV-2 *PLUS O* EIA and the licensed HIV-1/HIV-2 EIA.

Of the 1501 specimens tested, 14 were repeatedly reactive on both the GS HIV-1/HIV-2 *PLUS O* EIA and the licensed HIV-1 EIA, and confirmed as HIV-1 positive on a licensed Western Blot. As shown in Table 10, of the remaining 1487 prospective public health specimens not confirmed as positive for HIV-1 or HIV-2, 8 (0.54%) specimens were repeatedly reactive with the GS HIV-1/HIV-2 *PLUS O* EIA. In this sample population, seven (0.47%) specimens were repeatedly reactive with the licensed HIV-1/HIV-2 EIA. These 15 specimens (8 repeatedly reactive with the GS HIV-1/HIV-2 *PLUS O* EIA and 7 repeatedly reactive with the licensed HIV-1/HIV-2 EIA) were negative or indeterminate on a licensed HIV-1 Western blot and therefore tested with a licensed HIV-2 EIA. Of the 15 specimens tested with a licensed HIV-2 EIA, 1 was repeatedly reactive. This specimen was indeterminate when tested with an in-house HIV-2 Western blot.

Therefore, the GS HIV-1/HIV-2 *PLUS O* EIA detected all HIV-1 confirmed positives [14/14 (100%)] in this study of prospective public health populations in the U.S. and Canada.

Reactivity with HIV-1 Commercial Seroconversion Panels

The GS HIV-1/HIV-2 *PLUS O* EIA was tested with specimens from 50 commercially available seroconversion panels and compared to two FDA licensed HIV-1/HIV-2 EIAs and licensed HIV-1 Western blots. As shown in Table 11, the GS HIV-1/HIV-2 *PLUS O* EIA was equivalent [12/46* (26%)] or more sensitive [34/46* (74%)] when results were compared to one of the FDA licensed HIV-1/HIV-2 EIAs. The GS HIV-1/HIV-2 *PLUS O* EIA was equivalent [35/50 (70%)], more sensitive [9/50 (18%)], or less sensitive [6/50 (12%)] when results were compared to a second FDA licensed HIV-1/HIV-2 EIA. The GS HIV-1/HIV-2 *PLUS O* EIA was equivalent [13/50 (26%)] or more sensitive [37/50 (74%)] when compared to licensed HIV-1 Western blot results. Therefore, the GS HIV-1/HIV-2 *PLUS O* EIA was equivalent or better than licensed HIV-1/HIV-2 EIA tests and licensed HIV-1 Western blots for detection of antibody in HIV-1 seroconversion samples.

Table 11: Reactivity with 50 HIV-1 Commercial Seroconversion Panels

	HIV-1/HIV-2 *PLUS O* Equivalent	HIV-1/HIV-2 *PLUS O* More Sensitive	HIV-1/HIV-2 *PLUS O* Less Sensitive
vs. Licensed Kit #1	12/46* (26%)	34/46* (74%)	0 (0%)
vs. Licensed Kit #2	35/50 (70%)	9/50 (18%)	6/50 (12%)
vs. Licensed Western Blot	13/50 (26%)	37/50 (74%)	0 (0%)

*Four of the 50 seroconversion panels did not have test results with the licensed HIV-1/HIV-2 EIA Kit #1 and are no longer available for testing.

Reactivity with BBI Panels

When tested with four BBI Panels (Mixed Titer PRB203, Low Titer PRB105, African HIV Series AfrRB1, and World Wide WWRB301), 130/130 HIV positive members (100.0%) were reactive with the GS HIV-1/HIV-2 *PLUS O* EIA; 124/130 HIV positive members (95.4%) were reactive with an FDA licensed HIV-1/HIV-2 EIA; 128/130 HIV positive members (98.5%) were reactive with a second FDA licensed HIV-1/HIV-2 EIA.

Reactivity in Preselected Specimens from Individuals Positive for HIV-2 Antibodies and Confirmed by Western blot

A total of 302 specimens, obtained from HIV-2 confirmed antibody positive individuals, were tested with the GS HIV-1/HIV-2 *PLUS O* EIA. All specimens were repeatedly reactive with a licensed HIV-2 EIA and positive on an in-house HIV-2 Western blot. All of the 302 specimens tested were classified as repeatedly reactive with the GS HIV-1/HIV-2 *PLUS O* EIA for an estimated sensitivity in this study of 100% (95% confidence interval: 99.83% – 100%).

Reactivity in Specimens Known to be Positive for Antibodies to HIV-1 Group O

Seventy-seven different specimens known to be positive for antibodies to HIV-1 Group O (characterized by serotype and/or genotype) were tested with the GS HIV-1/HIV-2 *PLUS O* EIA. All [77/77 (100%)] of the HIV-1 Group O positive samples tested with the GS HIV-1/HIV-2 *PLUS O* EIA were initially or repeatedly reactive. (One sample was initially reactive and had insufficient volume for repeat testing.) The HIV-1 Group O sensitivity of the

GS HIV-1/HIV-2 *PLUS O* EIA in this study was 100%, with a 95% confidence interval of 99.35% to 100%.

Reactivity of Known Positives using the EVOLIS® Automated Microplate System

Comparative studies were performed with specimens known to be positive for HIV-1, HIV-2, and HIV-O antibody, including dilution series and seroconversion panels, using the EVOLIS® Automated Microplate System in comparison to manual testing.

A total of 100 known positive HIV-1 samples were tested once on each of three different instruments at each of three different sites. Approximately 60-80% of the known positive HIV-1 samples were diluted to be near the assay cut-off. For the 100 known positive HIV-1 samples combined across sites, 97.3% (292/300) of manual results were reactive; 100% (300/300) of the EVOLIS® Automated Microplate System results were reactive. The positive % agreement was 100% (292/292) with a 95% confidence interval range of 98.7%-100.0%.

A total of 100 known positive HIV-2 samples were tested once on each of three different instruments at each of three different sites. Approximately 60-80% of the known positive HIV-2 samples were near the assay cut-off. For the 100 known positive HIV-2 samples averaged across sites, 100% (100/100) of manual results were reactive; 100% (100/100) of the EVOLIS® Automated Microplate System results were reactive. The positive % agreement was 100% (100/100) with a 95% confidence interval range of 100.0%-100.0%.

The mean quantitative S/CO for the HIV-2 test panel was 10.95% lower on EVOLIS® as compared to manual testing. The difference was statistically significant, but not clinically significant.

These studies demonstrate comparable results for both methods when testing for HIV-1, HIV-2, and HIV-1 Group O antibodies with the GS HIV-1/HIV-2 *PLUS O* EIA.

Reactivity of Known Positives using the Elite™ Automated Microplate System

Comparative studies were performed with specimens known to be positive for HIV-1 group M, HIV-2, and HIV-1 group O antibody, including dilution series and seroconversion panels, using the Elite™ Automated Microplate System in comparison to manual testing.

A total of 100 known HIV-1 group M positive samples were tested once on each of three different instruments at each of three different sites. Approximately 60-80% of the known HIV-1 positive samples were diluted to be near the assay cut-off. All of these positive samples were reactive at all 3 sites, with both the Elite™ Automated Microplate System and manual testing. Averaged across all sites, the positive % agreement was 100% (100/100).

Performance of the Elite™ Automated Microplate System and manual testing were compared with 100 known HIV-2 positive samples that were tested once on each of three different instruments at each of three different sites. Approximately 60-80% of the known HIV-2 positive samples were near the assay cut-off. For the 100 known HIV-2 positive samples averaged across sites, 97% (97/100) of manual results were reactive and 100% (100/100) of the Elite™ Automated Microplate System results were reactive. The overall % agreement, averaged across all sites, was 97% (97/100).

A total of 50 known HIV-1 group O positive samples were tested once on each of three different instruments at each of three different sites. Approximately 40-60% of the known HIV-1 positive samples were low positives. Averaged across all 3 sites, 98% (49/50) of the Elite™ Automated Microplate System and 98% (49/50) of the manual testing results were reactive. The overall % agreement, averaged across all sites, was 100% (50/50).

These studies demonstrate comparable results for both methods when testing for HIV-1, HIV-2, and HIV-1 Group O antibodies with the GS HIV-1/HIV-2 *PLUS O* EIA.

Reactivity of Known Positives Using the ORTHO® Summit System

Head-to-head studies with dilution series, seroconversion series, and specimens known to be positive for HIV-1, HIV-2, and HIV-1 Group O antibody were performed using the ORTHO® Summit System with the ORTHO® Summit Sample Handling System* and the Bio-Rad manual equipment method of testing. Studies were also performed using the ORTHO® Summit Sample Handling System in comparison to the ORTHO VERSEIA® Pipetter. From these comparative studies, it was concluded that the GS HIV-1/HIV-2 *PLUS O* EIA assay results are acceptable for HIV-1, HIV-2, and HIV-1 Group O analytes using manual testing or using either of these ORTHO® pipetters with the ORTHO® Summit System.

Additional studies were performed with the stand-alone ORTHO VERSEIA® Pipetter and a stand-alone ORTHO® Summit Processor (OSP) in comparison with the ORTHO VERSEIA® Integrated Processor (VIP). These studies used dilution panels and known positive samples. With the HIV-1 dilution panels, the VIP showed an overall S/CO percent difference increase from the stand-alone system of 7.6%, and with the HIV-2 panels, the VIP showed an overall S/CO percent difference increase of 8.8%. For the HIV-1 known positive samples, the VIP showed a percent difference increase in S/CO of 7.1%, and for the HIV-2 known positive samples, the VIP showed a percent difference increase of 10.9%.

* The ORTHO® Summit Sample Handling System is now a legacy device no longer available for marketing.

14-PERFORMANCE CHARACTERISTICS OF CADAVERIC SPECIMEN TESTING

REPRODUCIBILITY

Inter-assay reproducibility of the GS HIV-1/HIV-2 *PLUS O* EIA was assessed using nineteen post-mortem sera and twenty normal donor sera, spiked with HIV-1 and HIV-2 positive serum to give reactivity near the cutoff. Each of the samples was tested once on three different days on each of three lots of the GS HIV-1/HIV-2 *PLUS O* EIA at one site. For inter-assay reproducibility over all lots, percent coefficient of variation (%CV) ranged from 5.84% to 22.37% for the post mortem samples, and from 4.84% to 21.44% for the normal donor samples.

SPECIFICITY

Specificity was evaluated in a clinical investigation at one site in three studies. In total, ninety-five (95) post-mortem samples and ninety-five (95) normal donor samples were tested concurrently on five lots of the GS HIV-1/HIV-2 *PLUS O* EIA. Repeatedly reactive specimens were additionally tested with a licensed HIV-1/HIV-2 EIA and confirmed with a licensed HIV-1 Western blot. Results are presented in Table 12 below.

Table 12: Reactivity with GS HIV-1/HIV-2 *PLUS O* EIA

Population	Number Tested	Nonreactive	Initially Reactive	Repeatedly Reactive	Confirmed Positive
Post-mortem	95	95 (100.0%)	0 (0.00%)	NA	NA
Normal Donor	95	94 (98.95%)	1* (1.05%)	1*	0

NA = Not Applicable

*This specimen was nonreactive by a licensed HIV-1/HIV-2 EIA and negative by HIV-1 Western Blot.

Specificity of the GS HIV-1/HIV-2 *PLUS O* EIA in testing cadaveric specimens was estimated by the following formula:

$$\frac{\text{(\# specimens - \# repeatedly reactive specimens)}}{\text{(\# specimens - \# repeatedly reactive specimens confirmed positive for HIV-1 or HIV-2)}} \times 100$$

A total of ninety-five (95) unselected post-mortem specimens were tested with the GS HIV-1/HIV-2 *PLUS O* EIA for determining specificity. All post-mortem specimens were compared to normal donor specimens. None of the post-mortem specimens were reactive with the GS HIV-1/HIV-2 *PLUS O* EIA. Thus, the GS HIV-1/HIV-2 *PLUS O* EIA has an estimated specificity of 100% (95% binomial confidence interval = [99.47%, 100%]). By comparison, one of the ninety-five normal donor specimens tested concurrently (1.05%) was initially and repeatedly reactive, but did not confirm positive for HIV-1 or HIV-2. The mean optical density for the 95 post-mortem samples was 0.054, whereas the mean for the 94 nonreactive normal donor samples was 0.044.

SENSITIVITY

Ninety-five (95) post-mortem samples and ninety-four (94) normal donor samples were pre-screened for antibody to HIV-1 and HIV-2 and found to be nonreactive. Each sample was divided into two portions. One portion of each post-mortem and normal donor sample was spiked at a potency near cutoff with a positive serum containing HIV-1 or HIV-2 antibody, and the remaining portion was left unspiked. The ninety-five spiked and unspiked post-mortem samples were tested concurrently with ninety-four spiked and unspiked normal donor specimens on the same run of the GS HIV-1/HIV-2 *PLUS O* EIA. Spiked specimens were expected to be reactive and therefore were not retested in duplicate. Results are presented in Table 13 below.

Table 13: Reactivity with GS HIV-1/HIV-2 *PLUS O* EIA

Population	Number Tested	Nonreactive	Initially Reactive	Repeatedly Reactive	Confirmed Positive
Spiked Post-mortem	95	0 (0.00%)	95 (100.0%)	NT	95 (100.0%)
Unspiked Post-mortem	95	95 (100.0%)	0 (0.00%)	NA	NA
Spiked Normal Donor	94	0 (0.00%)	94 (100.0%)	NT	94 (100.0%)
Unspiked Normal Donor	94	94 (100.0%)	0 (0.00%)	NA	NA

NT = Not Tested NA = Not Applicable

As can be seen in the table above, of ninety-five post-mortem samples and ninety-four normal donor samples, spiked at a potency near cutoff and tested concurrently, all (100.00%) were reactive with the GS HIV-1/HIV-2 *PLUS O* EIA (95% binomial

confidence interval = [99.47%, 100%]). These results demonstrate that the detection of HIV-1 and HIV-2 antibody in post-mortem samples is comparable to the detection in normal donors.

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