

# HUMAN IMMUNODEFICIENCY VIRUS TYPES 1 and 2

(Recombinant and Synthetic Peptides)

Genetic Systems™ HIV-1/HIV-2 *PLUS O* EIA

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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.

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For In Vitro Diagnostic Use

32588 • 480 Tests

32589 • 960 Tests

25256 • 4800 Tests

Lexicon/Lexique/Glosario/Glossar/Definizione				
English	Français	Español	Deutsch	Italiano
Wash Solution Concentrate (30X)	Solution de lavage concentrée	Solución concentrada para lavado	Konzentrierte Waschlösung	Soluzione di lavaggio concentrata
Chromogen: TMB Solution	Chromogène : solution de TMB	Cromógeno: solución de TMB	Chromogen: TMB-Lösung	Cromogeno: soluzione di TMB
Substrate Buffer	Tampon substrat	Tampón sustrato	Substratpuffer	Tampone substrato
Stopping Solution	Solution d'arrêt	Solución de parada	Stopplösung	Soluzione bloccante

WASH

TMB SOLUTION

SUB BUF

STOP

For Reference Use Only

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## 1 - NAME AND INTENDED USE

The Genetic Systems™ 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, plasma, and cadaveric serum specimens. It is indicated as a screening test for serum, plasma, and cadaveric serum specimens and as an aid in the diagnosis of infection with HIV-1 and/or HIV-2. The Genetic Systems™ HIV-1/HIV-2 *PLUS O* EIA is also intended for use with the Ortho Summit™ System (OSS) in the screening of blood donors.

## 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.<sup>1</sup> Additionally, transmission of these viruses can occur through tissue transplantation.<sup>2</sup> Human Immunodeficiency Virus Type 1 (HIV-1) has been isolated from patients with AIDS and AIDS-related complex (ARC).<sup>3-5</sup> 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.<sup>6-7</sup> Since the initial discovery, hundreds of cases of HIV-2 infection have been documented worldwide, including cases of AIDS related to HIV-2.<sup>8</sup> In the United States, there have been more than 80 cases of infection with HIV-2 reported, including two blood donors.<sup>9-14</sup>

This second immunodeficiency virus is similar to, but distinct from, HIV-1. Both viruses have similar morphology and lymphotropism,<sup>15</sup> and the modes of transmission appear to be identical.<sup>8,16</sup> 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.<sup>17</sup> 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.<sup>18</sup>

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).<sup>19-21</sup> Similarly, the HIV-2 strains have been classified into at least five subtypes (A through E).<sup>22</sup> 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.<sup>23</sup> 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.<sup>24,25</sup> The Genetic Systems™ 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.<sup>26-32</sup> 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 Genetic Systems™ HIV-1/HIV-2 *PLUS O* EIA must be retested in duplicate with the Genetic Systems™ 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 Genetic Systems™ 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 which 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

### Genetic Systems™ 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	<ul style="list-style-type: none"> <li>• Microwell plate with adsorbed purified HIV-1 and HIV-2 antigens.</li> <li>• ProClin® 150 preservative</li> </ul>	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)	<ul style="list-style-type: none"> <li>• Sodium chloride</li> <li>• Tween 20™</li> </ul>	Dilute 1:30 with deionized water. Clinical laboratory reagent water Type I or Type II is acceptable.
R3 • Specimen Diluent 1, 1, or 5 bottle(s) (100 ml)	<ul style="list-style-type: none"> <li>• Fetal calf serum</li> <li>• Buffer with protein stabilizers</li> <li>• 0.1% ProClin® 300 preservative</li> <li>• Sample indicator dye</li> </ul>	Use as supplied.
C0 • Negative Control 1, 1, or 5 vial(s) (8 ml)	<ul style="list-style-type: none"> <li>• Human serum; negative for HIV and HCV antibodies and HBsAg</li> <li>• 0.005% Gentamicin sulfate</li> <li>• 0.16% ProClin® 950 preservative</li> </ul>	Dilute in Specimen Diluent as described.
C1 • HIV-1 Positive Control 1, 2, or 10 vial(s) (1.4 ml)	<ul style="list-style-type: none"> <li>• Human HIV-1 antibody</li> <li>• Non-reactive for HBsAg and antibodies to HCV</li> <li>• 0.005% Gentamicin sulfate</li> <li>• 0.16% ProClin® 950 preservative</li> </ul>	Dilute in Specimen Diluent as described.
C2 • HIV-2 Positive Control 1, 2, or 10 vial(s) (1.4 ml)	<ul style="list-style-type: none"> <li>• Human HIV-2 antibody</li> <li>• Non-reactive for HBsAg and antibodies to HCV</li> <li>• 0.005% Gentamicin sulfate</li> <li>• 0.16% ProClin® 950 preservative</li> </ul>	Dilute in Specimen Diluent as described.
C3 • HIV-1 Group O Positive Control 1, 2, or 10 vial(s), (1.4 ml)	<ul style="list-style-type: none"> <li>• Rabbit HIV-1 Group O antibody in Human Serum, negative for HIV and HCV antibodies and HBsAg</li> <li>• 0.005% Gentamicin sulfate</li> <li>• 0.16% ProClin® 950 preservative</li> </ul>	Dilute in Specimen Diluent as described.
R4 • Conjugate Concentrate (11X) 1, 1, or 5 vial(s) (12 ml)	<ul style="list-style-type: none"> <li>• Purified HIV-1 and HIV-2 antigens labeled with peroxidase</li> <li>• Buffer with protein stabilizers</li> <li>• 0.005% Gentamicin sulfate</li> <li>• 0.5% ProClin® 300 preservative</li> <li>• Green dye</li> </ul>	Dilute in Conjugate Diluent as described.
R5 • Conjugate Diluent 1, 1, or 5 bottle(s), (120 ml)	<ul style="list-style-type: none"> <li>• Buffer with protein stabilizers</li> <li>• 0.1% ProClin® 300 preservative</li> </ul>	Use as supplied.
R8 • Substrate Buffer 1, 1, or 5 bottle(s) (120 ml)	<ul style="list-style-type: none"> <li>• Hydrogen peroxide</li> <li>• Citric acid/Sodium acetate buffer</li> <li>• DMSO</li> </ul>	Use as supplied.
R9 • Chromogen (11X) 1, 1, or 5 bottles(s) (12 ml)	<ul style="list-style-type: none"> <li>• Tetramethylbenzidine (TMB)*</li> </ul>	Dilute with Substrate Buffer as described.
R10 • Stopping Solution 1, 1, or ** bottle(s), (120ml)	<ul style="list-style-type: none"> <li>• 1N Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>)</li> </ul>	Use as supplied.
Plate Sealers	<ul style="list-style-type: none"> <li>• Clear plastic sealers</li> </ul>	Use as supplied.

\* Note: Tetramethylbenzidine is a non-carcinogenic and non-mutagenic chromogen for peroxidase.<sup>33,34</sup>

\*\* 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 strip 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 result in inaccurate test results.**

1. Wear 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. This test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with their potential hazards. Wear appropriate protective clothing, gloves and eye/face protection and handle appropriately with the requisite Good Laboratory Practices. 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. ProClin® 300 (0.1% or 0.5%) and ProClin® 950 (0.16%) are biocidal preservatives that are 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. The 1.0 N Sulfuric Acid ( $H_2SO_4$ ) Stopping Solution is irritating to skin and severely irritating or corrosive to eyes, 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 5-8 for non-hazardous disposal.
5. The Genetic Systems™ 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 *Universal Precautions* for bloodborne pathogens as defined by OSHA, the guidelines from the current CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories* 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, etc.), and wiped dry.<sup>35-38</sup>

Spills containing acid should be appropriately absorbed (wiped up) or neutralized, the area flushed with water and wiped dry; materials used to absorb the spill may require biohazardous waste disposal. Then the area should be decontaminated with one of the chemical disinfectants.

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.

## 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 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. Exercise care when opening vials and removing aliquots to avoid microbial contamination of the reagents.
  4. Use a clean, disposable container for the Conjugate Solution. Exposure of the Conjugate to sodium azide will result in its inactivation.
  5. 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.
  6. 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.
  7. Bring all reagents except Conjugate Concentrate to room temperature before use.
  8. 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.
  9. Handle the Negative Control and Positive Controls in the same manner as patient specimens.
  10. 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.

11. Inadequate adherence to package insert instructions may result in erroneous results.
12. Use only adequately calibrated equipment with this assay.
13. Use of dedicated equipment is recommended if equipment performance validations have not precluded the possibility of cross-contamination.
14. The Genetic Systems™ 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.
15. **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 10.)

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. 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 ( $\mu$ l)	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 Type I or Type II 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. 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 ( $\leq 37^{\circ}\text{C}$ ) 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 - GENETIC SYSTEMS™ 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  $\mu\text{l}$ , 1 ml, 10 ml, 25 ml and 50 ml, as needed (accurate within  $\pm 10\%$ ), and corresponding pipette tips; multichannel pipettors capable of delivering 25  $\mu\text{l}$  and 100  $\mu\text{l}$  are optional.
2. Appropriately sized graduated cylinders.
3. Dry-heat incubator capable of maintaining  $37 \pm 2^{\circ}\text{C}$ .

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:  $\pm (0.5\% + 0.005)$  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. An instrument without a reference filter can be used; however, areas in the bottoms of the wells that are opaque, scratched, or irregular may cause absorbance readings that are falsely elevated.
6. The Genetic Systems™ HIV-1/HIV-2 *PLUS O* EIA is approved for use with the Ortho™ Summit System and Ortho™ Summit Processor as a screening test for human serum and plasma specimens. The Genetic Systems™ HIV-1/HIV-2 *PLUS O* EIA Ortho® Assay Protocol Disk is available from Ortho-Clinical Diagnostics.
7. 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™.
8. Paper towels or absorbent pads for blotting.
9. Labeled null strips for testing partial plates.
10. 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.
11. Deionized or distilled water. Clinical laboratory reagent water Type I or Type II is acceptable.<sup>39</sup>
12. Gloves.

13. Laboratory timer.
14. EIA reagent reservoirs (optional).

### **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  $\leq 1$  hour.
2. Three Negative Controls, one HIV-1 Positive Control, one HIV-2 Positive Control, and one 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 provided 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.

### **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.
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  $\mu$ l of specimen in 50  $\mu$ l of Specimen Diluent and then transfer 100  $\mu$ l to the well), or diluted in-well (add 25  $\mu$ l of Specimen Diluent to each well first, followed by 75  $\mu$ l 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).**

15. Carefully remove the plate cover 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

<u>Sample Number</u>	<u>Absorbance</u>	<u>Total Absorbance</u>	=	<u>0.239</u>	=	0.080 (NCx)
1	0.075	3		3		
2	0.083					
3	<u>0.081</u>					
	0.239					

### Calculation of Results:

#### Cutoff Value

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

$$\text{NCx} = 0.080$$

$$\text{Cutoff Value} = 0.080 + 0.250 = 0.330$$

## 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 Genetic Systems™ 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 Genetic Systems™ HIV-1/HIV-2 PLUS O EIA. Initially reactive specimens should be retested in duplicate to validate the initial test results. 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. Reasons for non-repeatedly reactive specimens include:

- improper washing of microwell plates
- cross-contamination of non-reactive specimens with HIV-1 and/or HIV-2 Ab from a high titered specimen
- contamination of the Working TMB Solution by oxidizing agents (sodium hypochlorite, hydrogen peroxide, etc.)
- contamination of the Stopping Solution

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.

## **12-LIMITATIONS OF THE PROCEDURE**

1. The Genetic Systems™ 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 Genetic Systems™ 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 antibody-

ies to HIV-1 or HIV-2.<sup>40</sup> 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.<sup>40</sup> 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 serum 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.<sup>41,42</sup> However, in a prospective study, AIDS developed in 51% of homosexual men after 10 years of infection.<sup>43</sup>
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 Genetic Systems™ 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.<sup>44</sup> Borderline reactivity is more frequently non-specific, 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).<sup>45</sup>

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.<sup>46</sup>
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

Inter-assay and intra-assay reproducibility were determined by assaying a panel of 14 specimens consisting of 5 dilutions of an HIV-1 antibody positive sample (Group M); one HIV-1 (Group O) antibody positive sample; 6 dilutions of an HIV-2 antibody positive sample; and two undiluted HIV-negative samples. 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 at Bio-Rad Laboratories according to the National Committee for Clinical Laboratory Standards (NCCLS).<sup>a</sup> and <sup>b</sup> The mean Optical Density (OD), standard deviation (SD), and percent coefficient of variation (%CV) for each panel member are listed in Table 1 below.

**Table 1: Reproducibility of Genetic Systems™ HIV-1/HIV-2 PLUS O EIA**

Inter-assay Reproducibility					Intra-assay Reproducibility				
Panel Number	N*	Mean OD	SD <sup>a</sup>	%CV	Panel Number	N*	Mean OD	SD <sup>b</sup>	%CV
PP1	161	1.238	0.116	9.3%	PP1	161	1.238	0.101	8.2%
PP2	162	0.972	0.105	10.8%	PP2	162	0.972	0.077	8.0%
PP3	161	0.485	0.138	28.5%	PP3	161	0.485	0.049	10.2%
PP4	161	0.423	0.048	11.2%	PP4	161	0.423	0.042	10.0%
PP5	160	0.254	0.036	14.3%	PP5	160	0.254	0.019	7.3%
PP6	158	0.126	0.021	16.8%	PP6	158	0.126	0.009	7.1%
PP7	162	1.195	0.190	15.9%	PP7	162	1.195	0.106	8.9%
PP8	160	0.843	0.150	17.8%	PP8	160	0.843	0.066	7.8%
PP9	159	0.652	0.105	16.1%	PP9	159	0.652	0.057	8.7%
PP10	161	0.401	0.069	17.1%	PP10	161	0.401	0.035	8.7%
PP11	160	0.235	0.038	16.2%	PP11	160	0.235	0.021	8.8%
PP12	161	0.112	0.017	15.0%	PP12	161	0.112	0.011	10.0%
PP13	159	0.041	0.010	24.2%	PP13	159	0.041	0.004	9.9%
PP14	156	0.043	0.010	22.7%	PP14	156	0.043	0.005	11.9%

\* Outliers not included in statistical calculations

a. EP5-A Vol. 19 No. 2, p25 Equations C3 and C4    b. EP5-A Vol. 19 No. 2, p24 Equation C2

## SENSITIVITY AND SPECIFICITY

### 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 Genetic Systems™ HIV-1/HIV-2 PLUS O EIA are summarized in Tables 2 and 3. The data include serum and plasma samples obtained from donors at 3 geographically distinct locations, and 360 specimens from individuals with various medical conditions.

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

Results Obtained with Genetic Systems™ 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 Genetic Systems™ 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

<b>TOTAL: (Serum and Plasma)</b>	<b>11,159 (100.00%)</b>	<b>11,141 (99.84%)</b>	<b>18 (0.16%)</b>	<b>12 (0.11%)</b>	<b>0</b>	<b>0</b>
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As shown in Table 2, 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 Genetic Systems™ 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:

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

Thus, assuming a zero prevalence rate of antibodies to HIV-1 and HIV-2 in this population, the Genetic Systems™ HIV-1/HIV-2 *PLUS O* EIA had an estimated specificity in this study of  $(11,159 - 12) \times 100 / 11,159 = 99.89\%$  (95% confidence interval: 99.83 – 99.96).

### **Random blood donors tested with Ortho Summit™ System**

Additional specificity studies have been performed with the Genetic Systems™ HIV-1/HIV-2 *PLUS O* EIA using the Ortho Summit™ 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 Genetic Systems™ 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).

**Table 3: Detection of Antibodies to HIV-1 and/or HIV-2 in Individuals with Other Medical Conditions Unrelated to HIV Infection**

Results Obtained with Genetic Systems™ HIV-1/HIV-2 PLUS O EIA					Repeatedly Reactive Specimens	
Group	Number Tested	Non-Reactive	Initially Reactive	Repeatedly Reactive	HIV-2 EIA Repeatedly Reactive	Pos. by HIV-1 Immunoblot alone
Immunological diseases <sup>a</sup>	80 (100.00%)	78 (97.50%)	2 (2.50%)	2 (2.50%)	0	0
Acute or chronic viral diseases/ parasitic diseases <sup>b</sup>	200 (100.00%)	199 (99.50%)	1 (0.50%)	1 (0.50%)	0	0
Malignancies <sup>c</sup>	20 (100.00%)	20 (100.00%)	0 (0.00%)	0 (0.00%)	NA	NA
Miscellaneous / Other <sup>d</sup>	60 (100.00%)	59 (99.33%)	1 (1.67%)	1 (1.67%)	0	0
<b>TOTAL:</b>	<b>360</b> (100.00%)	<b>356</b> (98.89%)	<b>4</b> (1.11%)	<b>4</b> (1.11%)	<b>0</b>	<b>0</b>

- 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 Genetic Systems™ 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 Genetic Systems™ HIV-1/HIV-2 PLUS O EIA. There appears to be no correlation between reactivity in the Genetic Systems™ 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 Genetic Systems™ 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<sup>a</sup>) for whom the clinical status was unknown. The results of testing are shown in Table 4.

**Table 4: Reactivity in HIV-1 Known Positive Specimens**

Group	Genetic Systems™ 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 <sup>a</sup> )	199 (100%)	199 (100%)
TOTAL	1002 (100%)	1002 (100%)

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

Of the 313 diagnosed AIDS patients, 100% were repeatedly reactive with Genetic Systems™ 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 Genetic Systems™ 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

Genetic Systems™ 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 5. All specimens were screened with one or more FDA and/or Canadian licensed HIV-1/HIV-2 EIAs. All specimens repeatedly reactive with Genetic Systems™ 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 5: Reactivity in Specimens from High-Risk Individuals from the United States and Canada**

Group	No. Tested	Genetic Systems™ 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%) <sup>a</sup>	22 (2.9%) <sup>a</sup>	17 (2.3%) <sup>a</sup>
Canada	250	3 (1.2%) <sup>b</sup>	2 (0.8%) <sup>b</sup>	2 (0.8%) <sup>b</sup>
Total	1011	39 (3.9%)	24 (2.4%)	19 (1.9%)

- a. Seventeen (17) specimens were repeatedly reactive on both the Genetic Systems™ 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 Genetic Systems™ 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 Genetic Systems™ 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 Genetic Systems™ 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 Genetic Systems™ 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 US and one Canadian location. All specimens were tested with the Genetic Systems™ HIV-1/HIV-2 PLUS O EIA and an FDA licensed HIV-1/HIV-2 EIA. Specimens repeatedly reactive with Genetic Systems™ 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 Genetic Systems™ 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 6: Reactivity with Genetic Systems™ HIV-1/HIV-2 PLUS O EIA Prospective Public Health Specimens**

Results Obtained with Genetic Systems™ 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)	1501 - 14 1487	1474	27 - 14 13	22 - 14 8	21 - 14 7		
TOTAL:	1487 (100.00%)	1474 (99.13%)	13 (0.87%)	8 (0.54%)	7 (0.47%)		

\*This specimen, which was repeatedly reactive only on the Genetic Systems™ 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 Genetic Systems™ HIV-1/HIV-2 PLUS O EIA and the licensed HIV-1/HIV-2 EIA.

Of the 1,501 specimens tested, 14 were repeatedly reactive on both the Genetic Systems™ 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 6, of the remaining 1,487

prospective public health specimens not confirmed as positive for HIV-1 or HIV-2, 8 (0.54%) specimens were repeatedly reactive with the Genetic Systems™ 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 Genetic Systems™ 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 Genetic Systems™ 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 Genetic Systems™ 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 7, the Genetic Systems™ 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 Genetic Systems™ 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 Genetic Systems™ 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 Genetic Systems™ 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 7: Reactivity with 50 HIV-1 Commercial Seroconversion Panels**

	HIV-1/HIV-2 <i>PLUS O</i> Equivalent	HIV-1/HIV-2 <i>PLUS O</i> More Sensitive	HIV-1/HIV-2 <i>PLUS O</i> 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 Genetic Systems™ 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 Genetic Systems™ 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 Genetic Systems™ 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 Genetic Systems™ HIV-1/HIV-2 *PLUS O* EIA. All [77/77 (100%)] of the HIV-1 Group O positive samples tested with the Genetic Systems™ 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 Genetic Systems™ 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 Ortho Summit™ System**

Head-to-head studies with specimens known to be positive for HIV-1, HIV-2 and HIV-O antibody, dilution series and seroconversion series were performed using the Ortho Summit™ System and the Bio-Rad manual equipment method of testing. From these comparative studies, it was concluded that the Genetic Systems™ HIV-1/HIV-2 *PLUS* O EIA assay results are acceptable for HIV-1, HIV-2 and HIV-O analytes using either method of testing.

## **14-PERFORMANCE CHARACTERISTICS OF CADAVERIC SPECIMEN TESTING**

### **REPRODUCIBILITY**

Inter-assay reproducibility of Genetic Systems™ 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 Genetic Systems™ 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 Genetic Systems™ 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 8 below.

**Table 8: Reactivity with Genetic Systems™ 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 Genetic Systems™ HIV-1/HIV-2 PLUS O EIA in testing cadaveric specimens was estimated by the following formula:

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

A total of ninety-five (95) unselected post-mortem specimens were tested with the Genetic Systems™ 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 Genetic Systems™ HIV-1/HIV-2 PLUS O EIA. Thus, the Genetic Systems™ 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 Genetic

Systems™ 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 9 below.

**Table 9: Reactivity with Genetic Systems™ 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 Genetic Systems™ 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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