

# HUMAN IMMUNODEFICIENCY VIRUS TYPE 2

GS HIV-2 EIA

---

Enzyme Immunoassay (EIA) for the Detection of  
Antibody to Human Immunodeficiency Virus Type 2  
in Human Serum or Plasma

---

For *In Vitro* Diagnostic Use

32536

• 480 Tests

For Reference Use Only

<b>LEXICON</b>	
<b>WASH</b>	Wash Solution Concentrate (30X)
<b>STOP</b>	Stopping Solution

*For Reference Use Only*

# **CONTENTS**

- 1 - NAME AND INTENDED USE**
- 2 - SUMMARY AND EXPLANATION OF THE TEST**
- 3 - BIOLOGICAL PRINCIPLES OF THE PROCEDURE**
- 4 - REAGENTS**
- 5 - WARNINGS FOR USERS**
- 6 - PRECAUTIONS FOR USERS**
- 7 - REAGENT PREPARATION AND STORAGE**
- 8 - SPECIMEN COLLECTION, PREPARATION, AND STORAGE**
- 9 - GS HIV-2 EIA PROCEDURE**
- 10- QUALITY CONTROL - VALIDATION OF RESULTS**
- 11- INTERPRETATION OF RESULTS**
- 12- LIMITATIONS OF THE PROCEDURE**
- 13- PERFORMANCE CHARACTERISTICS**
- 14- BIBLIOGRAPHY**

## **1 - NAME AND INTENDED USE**

The GS HIV-2 EIA is an Enzyme Immunoassay for the detection of circulating antibodies to Human Immunodeficiency Virus Type 2 (HIV-2) in human serum or plasma and is indicated as an aid in the diagnosis of infection with Human Immunodeficiency Virus Type 2 (HIV-2).

## **2 - SUMMARY AND EXPLANATION OF THE TEST**

Human Immunodeficiency Virus Type 2 (HIV-2) is closely related to Human Immunodeficiency Virus Type 1 (HIV-1). HIV-1 has been isolated from patients with AIDS and AIDS-related complex (ARC)<sup>1-3</sup> and was the only virus recognized as a cause 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>4</sup> Since the initial discovery, more than 600 cases of HIV-2 infection have been documented worldwide, with over 40 cases of AIDS related to HIV-2.<sup>5</sup> In the United States, there have been sixty-two cases of infection with HIV-2 reported.<sup>6-9</sup>

This second immunodeficiency virus is similar to, but distinct from, HIV-1. The two viruses have very similar morphology and lymphotropism,<sup>10</sup> and the modes of transmission appear to be identical.<sup>5,11</sup> In addition, the HIV-1 and HIV-2 genomes exhibit about 60% homology in conserved genes such as gag and pol, and 30-40% homology in other, less conserved genes.<sup>12</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>13</sup>

Despite this immunologic cross-reactivity, detection of antibodies to HIV-2 with any of the licensed HIV-1 enzyme immunoassays (EIAs) is highly variable. In one study, detection of HIV-2 EIA positive samples ranged from 59.2% to 90.9%, depending on the test used.<sup>14</sup> The GS HIV-2 EIA was developed to detect antibodies to HIV-2.

Any specimen that reacts in an initial test (is initially reactive) with the GS HIV-2 EIA must be retested in duplicate with the GS HIV-2 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 specific antibodies to HIV-2, they may contain cross-reacting antibodies to HIV-1, or they may be non-specifically reactive. Therefore, additional, more specific or supplemental tests for antibody to both HIV-1 and HIV-2 such as immunoblot or radioimmunoprecipitation should be performed to facilitate interpretation of the results.

### **3 - BIOLOGICAL PRINCIPLES OF THE PROCEDURE**

The GS HIV-2 EIA is manufactured from virus that was isolated from the peripheral blood lymphocytes of a West African man by scientists at the Institut Pasteur in Paris, France.<sup>4</sup> The virus, which is designated HIV-2<sub>ROD</sub>, is propagated in a CEM cell line. The infected cell line is cultured and the virus is purified by centrifugation. The viral concentrate is disrupted and inactivated using a chaotropic agent and heat.

The purified, inactivated HIV-2 is adsorbed onto wells of a microwell plate. Samples to be tested are diluted in Specimen Diluent and added to each well, incubated with adsorbed antigen and washed. If antibodies to the virus are present, they bind to the antigen and are not removed by washing. The Conjugate Reagent, peroxidase-labeled goat anti-human immunoglobulin, is then added to the wells and will bind to the antibody-antigen complex, if present. Unbound Conjugate is removed by a wash step. Next, Chromogen/Buffered Substrate Reagent is added to the plate and allowed to incubate. A blue or blue-green color develops in proportion to the amount of antibody that has been bound to the antigen-coated plate. The enzyme reaction is stopped by the addition of acid. This will result in a color change to yellow. The optical absorbance of controls and specimens is determined with a spectrophotometer with wavelength set at 450 nm.

## 4 - REAGENTS

### GS HIV-2 EIA Product Description Product No: 32536 (480 Tests)

Component	Contents	Preparation
R1 • HIV-2 Coated Microwell Plates, 5 plates	<ul style="list-style-type: none"> <li>Microwell strips in holder, with adsorbed HIV-2 (Inactivated)</li> <li>Thimerosal</li> </ul>	Use as supplied.
C0 • HIV-2 EIA Negative Control, 1 vial (0.8 mL)	<ul style="list-style-type: none"> <li>Human serum/plasma</li> <li>Non-reactive for HBsAg and antibodies to HIV-1, HIV-2 and HCV</li> <li>0.1% Sodium Azide</li> <li>0.01% Thimerosal</li> </ul>	Dilute in Specimen Diluent as described.
C1 • HIV-2 EIA Positive Control, 1 vial (0.8 mL)	<ul style="list-style-type: none"> <li>Human serum / plasma containing HIV-2 immunoglobulin</li> <li>Heat inactivated</li> <li>Non-reactive for HBsAg and antibodies to HCV</li> <li>0.1% Sodium Azide</li> <li>0.01% Thimerosal</li> </ul>	Dilute in Specimen Diluent as described.
R2 • Wash Solution Concentrate (30X), 2 bottles (120 mL)	<ul style="list-style-type: none"> <li>Sodium Chloride</li> <li>Tween 20</li> </ul>	Dilute to working dilution with deionized water.
R3 • HIV-2 EIA Conjugate Concentrate, 1 vial (1.5 mL)	<ul style="list-style-type: none"> <li>Goat anti-human IgM and IgG horseradish peroxidase conjugated solution</li> <li>0.01% Thimerosal</li> </ul>	Dilute with Conjugate Diluent as described.
R4 • HIV-2 EIA Conjugate Diluent, 1 bottle (120 mL)	<ul style="list-style-type: none"> <li>Normal goat serum</li> <li>0.01% Thimerosal</li> </ul>	Use as supplied.
R5 • HIV-2 EIA Specimen Diluent Concentrate (10X), 1 bottle (120 mL)	<ul style="list-style-type: none"> <li>Diluent for specimen</li> <li>0.1% Thimerosal</li> </ul>	Dilute with deionized water as described.
R6 • EIA Chromogen Reagent, 1 vial (1.5 mL)	<ul style="list-style-type: none"> <li>Tetramethylbenzidine (TMB)*</li> <li>Dimethylsulfoxide (DMSO)</li> </ul>	Dilute with EIA Buffered Substrate as described.
R7A • EIA Buffered Substrate, 1 bottle (120 mL)	<ul style="list-style-type: none"> <li>Hydrogen Peroxide</li> <li>Citric Acid</li> <li>Phosphate Buffer</li> </ul>	Use as supplied.
R10 • Stopping Solution 1 bottle (120 mL)	<ul style="list-style-type: none"> <li>1N H<sub>2</sub>SO<sub>4</sub></li> </ul>	Use as supplied.
1 Pad Plate Sealers, 25 sealers each	<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>15,16</sup>

Store the kit at 2-8°C. Bring all reagents except HIV-2 EIA Conjugate Concentrate to room temperature (15-30°C) before use. Return all reagents to 2-8°C immediately 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 and plasma 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. 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 protective clothing, including lab coat, eye/face protection and disposable gloves (synthetic, non-latex gloves are recommended) while handling kit reagents and patient samples. 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 kit components (refer to REAGENTS section):
  - a. (0.01% or 0.1%) Thimerosal (Merthiolate Sodium), an Organo-Mercury biocidal preservative that targets the central nervous system (CNS), is a reproductive toxicant and significant sensitizer. Prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals; there are ample cases of sensitization resulting from exposure to dilute Thimerosal solutions. Avoid release to the environment, danger of cumulative effects. Spent mercury-containing solutions with a concentration greater than 0.2 ppm must be disposed of as US Federal RCRA hazardous waste (D009), however, dispose of all wastes in accordance with local, regional, and national regulations. (Note: mercury (Hg) makes up 49.55% of the Thimerosal

molecule, thus a component with 0.01% Thimerosal contains ~0.005% (~50 ppm) mercury w/v). In case of contact with skin, wash immediately with plenty of water.

- b. 0.1% Sodium Azide ( $\text{NaN}_3$ ) is a toxic biocidal preservative which has been evident to kill at low concentrations if enough is ingested. Sodium Azide may react with certain metals, including lead or copper often found in plumbing, to form highly explosive metal azides. If solutions containing dilute azide are disposed of in the sink after inactivation, flush with copious water to prevent potential explosive build-up.
  - c. The EIA Chromogen Reagent contains concentrated Dimethyl Sulfoxide (DMSO), a skin and eye irritant that readily penetrates skin and is capable of carrying other chemicals or materials into the body. It is recommended that nitrile, neoprene or other non-latex synthetic gloves be used when handling it, as DMSO penetrates latex immediately, possibly carrying potentially sensitizing latex molecules into the body.
  - d. The 1.0N Sulfuric Acid ( $\text{H}_2\text{SO}_4$ ) 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 6-9 for non-hazardous disposal.
5. 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 patho-

gens 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) has been tested and found non-reactive for Hepatitis B surface antigen (HBsAg), and antibodies to Hepatitis C virus (HCV) and human immunodeficiency viruses (HIV-1 and HIV-2).
  - b. Human source material, containing HIV-2 immunoglobulin, used in the preparation of the Positive Control (C1) has been heat-treated. It has been tested and found nonreactive for Hepatitis B surface antigen (HBsAg) and antibodies to Hepatitis C virus (HCV).
  - c. The adsorbed virus (microplate, R1) is inactivated using a chaotropic agent and heat.
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>17-19</sup>

Spills containing acid should be appropriately absorbed (wiped up) or neutralized and wiped dry. Then the area should be decontaminated with one of the chemical disinfectants. Materials 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.<sup>19,20</sup>

## 6 - PRECAUTIONS FOR USERS

1. Do not use the kit beyond the expiration date.
2. The only reagents that may be used with different lots of the GS HIV-2 EIA are the EIA Chromogen Reagent, EIA Buffered Substrate, Wash Solution Concentrate and Stopping Solution. Do not mix any other reagents from different lots.
3. Do not use the EIA Buffered Substrate for the EIA Chromogen Diluent in other Bio-Rad tests.
4. 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:
  - Wash Solution Concentrate (R2) - Catalog # 25261
  - Stopping Solution (R10) - Catalog # 25260
5. Exercise care in opening and removing aliquots from vials to avoid microbial contamination.
6. Use a clean, disposable container for Conjugate Reagent. Exposure of Conjugate Reagent to sodium azide or serum will inactivate Conjugate Reagent.
7. Avoid exposing EIA Chromogen Reagent or Chromogen/Buffered Substrate Reagent to strong light during storage or incubation. Do not allow the chromogen solutions to come into contact with an oxidizing agent. Do not vortex the EIA Chromogen Reagent or the Chromogen/Buffered Substrate Reagent.
8. Use clean, plastic disposable containers to prepare and store the Chromogen/Buffered Substrate Reagent. 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.

## 7 - REAGENT PREPARATION AND STORAGE

### Working Specimen Diluent

The HIV-2 EIA Specimen Diluent Concentrate should be thoroughly mixed before preparation of the working strength dilution. The Working Specimen Diluent (1X) is a 1:10 dilution of the HIV-2 EIA Specimen Diluent Concentrate (10X) provided with the kit. Prepare Working Specimen Diluent (1X) as needed by mixing one part concentrate (10X) with nine parts deionized water. Label container with lot number of concentrate and date of preparation. The Working Specimen Diluent (1X) may be stored in the refrigerator (2-8°C) for up to two weeks. It should be brought to room temperature and mixed thoroughly prior to use.

### Working Conjugate Solution

Bring Conjugate Diluent to room temperature. Invert Diluent and Conjugate Concentrate to mix before using. Prepare a 1:101 dilution for each strip to be tested by mixing 10 µL of Conjugate Concentrate to 1 mL of Conjugate Diluent. Note Concentrate lot number, date and time of preparation on container. Mix Working Solution when combined and again just prior to use. Working Solution is stable for 8 hours.

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

Do not add all the Concentrate to Diluent. Prepare only the amount of reagent required, according to the following table:

#### 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)	10	20	30	40	50	60	70	80	90	100	110	120
Amount of Conjugate Diluent (mL)	1	2	3	4	5	6	7	8	9	10	11	12

\* Complete Plate

## Working Chromogen Solution

Bring Chromogen Reagent and Buffered Substrate to room temperature. Invert the Chromogen Reagent and Buffered Substrate to mix before using. Prepare a 1:101 dilution for each strip to be tested by mixing 10  $\mu\text{L}$  of Chromogen Reagent to 1 mL of Buffered Substrate. Note Chromogen Reagent lot number, date and time of preparation on container. Mix Working Solution gently when combined and again just prior to use. Working Chromogen Solution should be kept in the dark and used within 8 hours.

Chromogen Reagent may be in crystalline form at refrigerator temperature and should be allowed to liquefy to room temperature prior to use. Chromogen Reagent should be colorless to slightly yellow. Any other color indicates that the reagent is contaminated and should not be used.

The Working Chromogen Solution should be colorless. A distinct blue color indicates that the reagent is contaminated. Discard the Working Chromogen Solution and prepare fresh reagent in a clean container.

Do not add all the Chromogen Reagent to Buffered Substrate. Prepare only the amount of Solution required, according to the following table:

### Preparation of the Working Chromogen Solution by Strip

Number of Strips to be used	1	2	3	4	5	6	7	8	9	10	11	12*
Amount of EIA Chromogen Reagent ( $\mu\text{L}$ )	10	20	30	40	50	60	70	80	90	100	110	120
Amount of Buffered Substrate (mL)	1	2	3	4	5	6	7	8	9	10	11	12

\* Complete Plate

## Working Wash Solution

Prepare Working Wash Solution as needed by adding one part Wash Solution Concentrate (30X) to 29 parts of deionized 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. Note lot number and date prepared on the container.

## 8 - SPECIMEN COLLECTION, PREPARATION AND STORAGE

Serum or plasma may be used in the test. Specimens with observable particulate matter should be clarified by centrifugation prior to testing. Grossly hemolyzed, lipemic, or icteric specimens may be compromised and should be redrawn.

Specimens may be stored at 2-8°C for one week. For long-term storage, the specimens should be frozen (at -15°C or colder). Avoid multiple freeze-thaw procedures. If specimens are to be shipped, they should be packed in compliance with Federal Regulations covering the transportation of etiologic agents.

## 9 - GS HIV-2 EIA PROCEDURE

**Note: Once this assay has been started, it should be completed without interruption. Use a separate disposable tip for each specimen to prevent cross-contamination.**

### Materials Required

See REAGENTS Section on page 6.

### Materials Required But Not Provided

1. Precision pipettes to deliver 5 µL, 10 µL, 100 µL, 1 mL, 5 mL, and 10 mL (accurate within ± 10%) or automated pipettor-dilutor.
2. Appropriate containers to prepare diluted specimens and reagents.
3. Dry-heat incubator capable of maintaining 37 ± 1°C.
4. Microwell plate or strip washer qualified for use with this assay.

5. Microwell plate or 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)

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. 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™ (West Chemical Products, Inc.).
7. Paper towels or absorbent pads for blotting.
8. Labeled null strips, for testing partial plates.

## EIA Procedure

1. **Bring all of the reagents except the HIV-2 Conjugate Concentrate to room temperature** before beginning the assay procedure.
2. Prepare Working Specimen Diluent, Working Wash Solution, Working Conjugate Solution, and Working Chromogen Solution. See Reagent Preparation section. Mix gently when combined and again just before use.
3. Remove strips not needed for the assay and replace them with labeled Null Strips, if necessary.
4. 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.

5. If sample identity is not maintained by automatic procedure, label or identify the individual wells for each specimen and control on a data sheet.
6. **Dilute specimens, HIV-2 Positive Control, and HIV-2 Negative Control 1:401** in Working Specimen Diluent (for example 5  $\mu$ L specimen plus 2.0 mL Working Specimen Diluent). Mix thoroughly. Avoid foaming of the Diluent. Assay two Positive Control and three Negative Control dilutions with each plate or partial plate of specimens. Controls and specimens must be subjected to the same reagents, processes and incubation times.
7. **Add 100  $\mu$ L of the diluted specimen or control to the appropriate well.**
8. Cover the microwell plate with a plate sealer or use other means to minimize evaporation and **incubate the plate for 60 to 65 minutes at  $37^{\circ} \pm 1^{\circ}\text{C}$ .**
9. At the end of the incubation period, carefully remove the plate sealer, if used, and **aspirate** the fluid from each well into a biohazard container. **Wash the microwell plate or strip a minimum of five times** with Working Wash Solution (at least 300  $\mu$ L/well/wash). Aspirate the Wash Solution after each wash. After the last wash, aspirate liquid completely, or 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.
10. **Add 100  $\mu$ L of Working Conjugate Solution to each well containing a specimen or control.**
11. Cover the microwell plate with a fresh plate sealer, or use other means to minimize evaporation, and **incubate the plate for 60 to 65 minutes at  $37^{\circ} \pm 1^{\circ}\text{C}$ .**

12. At the end of the incubation period, carefully remove the plate sealer, if used, and **aspirate** the fluid in each well into a bio-hazard container. **Wash the plates a minimum of five times** with Working Wash Solution (at least 300  $\mu\text{L}$ /well/wash). Aspirate the Wash Solution after each wash. After the last wash, aspirate liquid completely, or 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.
13. **Add 100  $\mu\text{L}$  of Working Chromogen Solution per well.** Cover the microwell plate with a fresh plate sealer, or use other means to minimize evaporation, and **incubate plates in the dark for 30 to 33 minutes at room temperature.** For example, cover the plates with black plastic or place in a drawer.
14. Carefully remove the plate sealer, if used, and **add 100  $\mu\text{L}$  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.**
15. **Read absorbance within 30 minutes** after adding the Stopping Solution using a 450 nm filter with 615 nm to 630 nm as the reference. Blank on air. Check to ensure that all strips have been pressed firmly into place before reading.

### **Decontamination**

Decontaminate the waste solution by bringing the disinfectant concentration to  $\geq 0.5\%$  sodium hypochlorite (10% household bleach), 70% ethanol, or 0.5% Wescodyne.™

Upon completion of the assay, aspirate the liquid (acid) from the wells of the microwell plate and neutralize the acid. **DO NOT USE THE MICRO-WELL WASHER TO ASPIRATE ACID AND DO NOT ASPIRATE ACID INTO BLEACH.** The neutralized solution can then be autoclaved or decontaminated. Handle all material as if capable of transmitting infectious disease.

## 10-QUALITY CONTROL - VALIDATION OF RESULTS

The presence or absence of antibody to HIV-2 is determined by relating the absorbance value of the specimen to the cutoff value. The cutoff value is determined by adding 0.150 to the mean absorbance value of the Negative Controls.

### Mean Negative Control absorbance value (NCX)

Determine the mean of the Negative Controls as shown in the example below:

Negative Control Sample Number	Absorbance	Total Absorbance	=	<u>0.195</u>	=	0.065 (NCX)
1	0.064	3				3
2	0.055					
3	<u>0.076</u>					
	0.195					

The individual Negative Control absorbance values must be greater than 0.000 AU and less than or equal to 0.140 AU. One Negative Control absorbance value may be discarded if it is outside this range. The NCX may be calculated from the two remaining values.

### Mean Positive Control absorbance value (PCX)

Determine the mean of the Positive Controls as shown in the example below:

Positive Control Sample Number	Absorbance	Total Absorbance	=	<u>2.936</u>	=	1.468 (PCX)
1	1.435	2				2
2	<u>1.501</u>					
	2.936					

The PCX must be greater than or equal to 0.700 AU, and each Positive Control absorbance value must be within the range of 0.65 to 1.35 times the PCX. No Positive Control absorbance value may be discarded.

Both of the Positive Control absorbance values above are within the range of 0.65 to 1.35 times the PCX as shown by the following calculation.

$$0.65 \times (\text{PCX}) = 0.65 \times 1.468 = 0.954$$

$$1.35 \times (\text{PCX}) = 1.35 \times 1.468 = 1.982$$

Therefore, the acceptable range in the above example is 0.954 to 1.982.

### **Cutoff Value**

The cutoff value is the NCX plus 0.150.

Example:

$$\text{NCX} = 0.065$$

$$\text{Cutoff Value} = 0.065 + 0.150 = 0.215$$

### **Validity Criteria**

A run is valid if the individual absorbance units (AU) of the Negative Control are greater than 0.000 AU and equal to or less than 0.140 AU. One Negative Control absorbance value may be discarded. Also the mean absorbance value of the Positive Controls (PCX) must be greater than or equal to 0.700 AU and the individual Positive Control absorbance values must be within the range of 0.65 to 1.35 times the PCX. No Positive Control absorbance value may be discarded.

If the Positive and Negative Controls are not within the acceptance range, technique or reagents should be suspected and the plate(s) should be repeated.

## 11-INTERPRETATION OF RESULTS

1. Specimens with absorbance values less than the cutoff value are considered non-reactive by the criteria of the GS HIV-2 EIA and may be considered negative for antibody to HIV-2. Further testing is not required.
2. An absorbance value of less than 0.000 AU may indicate a procedural or instrument error which should be evaluated. That result is invalid and that specimen must be re-run.
3. Specimens with absorbance values greater than or equal to the cutoff value are considered reactive (initially reactive) by the criteria of the GS HIV-2 EIA, but before interpretation, the sample should be retested in duplicate. If either duplicate retest is reactive, the specimen is considered repeatedly reactive.
4. Initially reactive specimens that do not react in either of the duplicate repeat tests are considered negative for antibodies to HIV-2.
5. If the specimen is repeatedly reactive, the probability that antibodies to HIV-2 are present is high, especially in specimens obtained from subjects at increased risk for HIV-2 infection or in specimens with very high absorbance values. In some instances, specimens that contain antibodies to HIV-1 may also be repeatedly reactive in the GS HIV-2 EIA. Repeatedly reactive specimens should be tested by additional, more specific or supplemental tests, such as immunoblot or radioimmunoprecipitation, for antibody to both HIV-1 and HIV-2.

Specimens that are repeatedly reactive by the GS HIV-2 EIA and are found to be positive by additional, more specific or supplemental testing for HIV-2 but negative or indeterminate (lacking glycoprotein reactivity) for HIV-1 are considered to be specific for antibodies to HIV-2. Specimens that are repeatedly reactive by the GS HIV-2 EIA and are found to be negative or indeterminate by additional, more specific or sup-

plemental testing for HIV-2 but positive for HIV-1 are considered to be positive for antibodies to HIV-1.

Specimens that are repeatedly reactive by the GS HIV-2 EIA and are found to be positive by additional, more specific or supplemental testing for both HIV-2 and HIV-1 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 repeatedly reactive by the GS HIV-2 EIA and negative or indeterminate on additional, more specific testing for antibodies to both HIV-1 and HIV-2 is unclear. Clarification may be obtained by testing another specimen taken three to six months later.

## 12-LIMITATIONS OF THE PROCEDURE

1. The GS HIV-2 EIA Procedure and the Interpretation of Results must be followed closely when testing for the presence of antibodies to HIV-2 in plasma or serum from individual subjects. Data regarding the interpretation were derived from testing serum or plasma samples. Insufficient data are available to interpret tests performed on other body specimens, pooled blood or processed plasma, and products made from such pools; testing of these specimens is not recommended.
2. Most specimens (50-90%) that are positive for antibody to HIV-1 will also react in the GS HIV-2 EIA due to cross-reactivity primarily between the core (*gag*) and polymerase (*pol*) proteins of the two viruses. Samples with repeatedly reactive test results must be investigated further by additional more specific or supplemental tests, such as immunoblot or radioimmunoprecipitation, for antibodies to both HIV-1 and HIV-2.
3. The GS HIV-2 EIA detects circulating antibodies to HIV-2 and thus is useful in evaluating patients with signs or symptoms of AIDS, and in establishing prior infection with HIV-2. Clinical studies continue to clarify and refine the interpretation and

medical significance of the presence of antibodies to HIV-2.<sup>5</sup> Repeatedly reactive specimens must be investigated by additional, more specific, or supplemental tests. A person who is confirmed to have antibodies to HIV-2 by additional, more specific testing, such as immunoblot or radioimmuno-precipitation, is presumed to be infected with the virus, and appropriate counseling and medical evaluation should be offered. Such an evaluation should be considered an important part of testing for antibody to HIV-2 and should include confirmation of the test result on a freshly-drawn sample.

4. AIDS and AIDS-related conditions are clinical syndromes, and their diagnosis can only be established clinically.<sup>21</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-2 is present.
5. 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-2.
6. The risk of an asymptomatic person with a repeatedly reactive serum developing AIDS or an AIDS-related condition is not known.<sup>5</sup>
7. Data obtained from testing persons both at increased and at low risk for HIV-2 infection suggest that repeatedly reactive specimens with high reactivity on the GS HIV-2 EIA may be more likely to demonstrate the presence of the HIV-2 antibodies by additional, more specific, or supplemental testing. Borderline reactivity is more frequently nonspecific, especially in samples obtained from persons at low risk for infection with HIV-2. Borderline reactivity in the GS HIV-2 EIA may also be indicative of infection with HIV-1.
8. An absorbance value of less than 0.000 AU may indicate a procedural or instrument error which should be evaluated. That result is invalid and that specimen must be re-run.

## 13-PERFORMANCE CHARACTERISTICS

### Reproducibility

Inter-assay reproducibility was determined by assaying seven specimens, along with the HIV-2 EIA Positive and HIV-2 EIA Negative Controls, at each of six different sites. Three lots of material were used. Intra-assay reproducibility was determined by assaying replicates of the HIV-2 EIA Positive and HIV-2 EIA Negative Control as well as one positive sample. Three lots of material were also used in this testing, and the resultant reproducibility was analyzed by applying the pooled estimate of standard deviation. Results of reproducibility testing are shown in Table 1 below.

**Table 1: Reproducibility of the GS HIV-2 EIA**

Inter-assay Reproducibility				
Specimen	N	Mean OD/CO	SD	%CV
1	36	4.927	1.010	20.5%
2	36	3.530	0.789	22.4%
3	36	2.799	0.897	32.0%
4	36	2.366	0.449	19.0%
5	36	1.821	0.401	22.0%
6	18	0.838	0.279	33.3%
7	16	0.205	0.056	27.3%
HIV-2 EIA Positive Control	276	1.228*	0.226	18.4%
HIV-2 EIA Negative Control	412	0.063*	0.018	28.6%
Intra-assay Reproducibility				
Specimen	N	Mean OD	SD	%CV
HIV-2 EIA Positive Control	288	0.900	0.040	4.4%
HIV-2 EIA Negative Control	288	0.060	0.008	13.3%
Positive Sample	288	0.585	0.028	4.8%

\* Controls are expressed as mean OD values.

# SPECIFICITY AND SENSITIVITY

## Specificity Studies

### Reactivity in Blood Donors and Individuals with Other Medical Conditions Unrelated to HIV-2 Infection

The results of testing on specimens obtained from blood donors and specimens from individuals with medical conditions unrelated to HIV-2 infection are summarized in Table 2 below. The data include 6,022 serum samples (sites 1 and 2) and 2,001 plasma samples (site 3) obtained from donors at three geographically distinct locations, and 189 specimens from individuals with various medical conditions.

**Table 2: Detection of Antibodies to HIV-2 in Blood Donors and Individuals with Other Medical Conditions Unrelated to HIV-2 Infection**

Group	Results Obtained with GS HIV-2 EIA				Repeatedly Reactive Specimens	
	Number Tested	Non-Reactive	Initially Reactive	Repeatedly Reactive	Positive by HIV-2 Immunoblot <sup>g</sup>	Positive by HIV-1 Immunoblot <sup>h</sup>
Blood donors, Site 1	4,022 (100%)	4,015 (99.8%)	7 (0.2%)	6 (0.1%)	0 (0%)	4 (0.1%)
Blood donors, Site 2	2,000 (100%)	1,985 (99.2%)	15* (0.8%)	9 (0.4%)	0 (0%)	1 (0.1%)
Blood donors, Site 3	2,001 (100%)	1,994 (99.5%)	10 (0.5%)	6 (0.3%)	0 (0%)	1 (0.1%)
Bacterial/Parasitic diseases <sup>a</sup>	5 (100%)	5 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Autoimmune diseases <sup>b</sup>	65 (100%)	65 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Antibody to HTLV-I <sup>c</sup>	43 (100%)	41 (95.3%)	2 (4.7%)	0 (0%)	0 (0%)	0 (0%)
Other viral diseases <sup>d</sup>	30 (100%)	30 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Malignancies <sup>e</sup>	30 (100%)	30 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Multiple transfusions	6 (100%)	6 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Other <sup>f</sup>	10 (100%)	10 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

\* One initially reactive specimen was not available for repeat testing.

<sup>a</sup> 5 toxoplasmosis

- b 11 Rheumatoid factor positive, 10 systemic lupus erythematosus, 2 anti-nuclear antibody, 11 Sjogren's syndrome, 4 Ankylosing Spondylitis, 12 asthma and 15 other autoimmune diseases including crest syndrome, polymyositis, Bechet's syndrome, vasculitis, Reynaud's syndrome and polymyalgia.
- c Positive (by Western blot and/or RIPA) for both envelope (gp46 or gp68) and core (p24) antigens.
- d 5 Cytomegalovirus, 5 Herpes Simplex virus, 5 Epstein-Barr virus, 5 Rubella, 7 Hepatitis B surface antigen and 3 antibody to Hepatitis B core antigen.
- e 5 prostate cancer, 4 breast cancer, 5 cervical cancer, 1 lung cancer, 5 colon cancer, 5 leukemia, 2 lymphoma, and 3 myeloma.
- f 5 icteric specimens, and 5 lipemic specimens.
- g Specimens were considered positive by HIV-2 immunoblot if two of the following three bands were present: gp140, gp36, or p26.
- h Specimens were considered positive by HIV-1 immunoblot if two of the following three bands were present: gp160/120, gp41, or p24.

As shown above, 99.6% of the blood donor population were initially non-reactive, 0.4% were initially reactive and 0.3% were repeatedly reactive. Twenty one (65.6%) of the 32 initially reactive specimens were repeatedly reactive upon retesting. Seven of the 21 specimens that were repeatedly reactive with the GS HIV-2 EIA were also reactive with a licensed HIV-1 EIA and six were classified as positive by HIV-1 immunoblot; the remaining 14 specimens were not reactive by HIV-1 EIA. None of the repeatedly reactive specimens was positive for antibodies for HIV-2 by immunoblot, although 17 were indeterminate by HIV-2 immunoblot.

Two specimens (4.7%) from individuals positive for antibody to HTLV-I were initially reactive in the GS HIV-2 EIA. Neither specimen was repeatedly reactive by the GS HIV-2 EIA, nor positive by HIV-2 immunoblot.

### **Cross-Reactivity in Specimens from Patients and High-Risk HIV-1 Seropositive Individuals**

The cross-reactivity of the GS HIV-2 EIA was determined by testing serum and plasma samples from patients diagnosed as having AIDS (n=21), ARC (n=19), or lymphadenopathy syndrome (LAS: n=77), and from members of other high risk groups, including homosexual males (HM: n=144), IV drug users (IVDU: n=6), and individuals with miscellaneous risk factors (Other: n=6). The results of HIV-2 testing are shown in Table 3.

**Table 3: Cross-Reactivity in Specimens from Patients and High-Risk HIV-1 Seropositive Individuals**

Group	Tested	Non-Reactive	Initially Reactive	Repeatedly Reactive <sup>a</sup>
AIDS	21 (100%)	11 (52.4%)	10 (47.6%)	8/19 (42.1%)
ARC	19 (100%)	10 (52.6%)	9 (47.4%)	7/17 (41.2%)
LAS	77 (100%)	23 (29.9%)	54 (70.1%)	44/68 (64.7%)
HM	144 (100%)	44 (30.6%)	100 (69.4%)	93/139 (66.9%)
IVDU	6 (100%)	1 (16.7%)	5 (83.3%)	5/6 (83.3%)
Other <sup>b</sup>	6 (100%)	1 (16.7%)	5 (83.3%)	5/6 (83.3%)
Total:	273 (100%)	90 (33.0%)	183 (67.0%)	162/255 (63.5%)

<sup>a</sup> Some of the initially reactive specimens were not available for repeat testing. In this column, the numerator reflects the number of initially reactive specimens in each risk group that were retested and found to be repeatedly reactive. The denominator reflects the total number of specimens in each group minus the number of initially reactive specimens, in that category, that were not available for retesting. The percent of specimens that were repeatedly reactive was then calculated from these values.

<sup>b</sup> 2 sex partners of high-risk group members; 1 individual who traveled in Africa; 1 individual with multiple heterosexual partners (non-reactive in the GS HIV-2 EIA); 1 blood product recipient; 1 individual with unspecified risk factors.

One hundred sixty two (98.2%) of the 165 HIV-1 seropositive specimens that were initially reactive by GS HIV-2 EIA, and were available for retesting, were repeatedly reactive by GS HIV-2 EIA. Cross-reaction of the GS HIV-2 EIA on HIV-1 antibody-positive subjects was detected in 41.2% - 83.3% of cases, with an average of 63.5% detected.

One hundred and forty of the 162 repeatedly reactive specimens were tested by HIV-2 immunoblot.\* Forty of the 140 repeatedly reactive specimens tested by immunoblot were classified positive, 99 were classified indeterminate, and one was classified negative for antibodies for HIV-2 by additional, more specific or supplemental testing. All 40 HIV-2 immunoblot positive specimens were also classified positive for antibodies to HIV-1 by HIV-1 immunoblot.

These findings illustrate the fact that immunoblots for HIV-1 and HIV-2 cannot distinguish type-specific antibodies in many cases.

Given the low prevalence of HIV-2 infection in the United States, it is assumed that specimens that are positive for both HIV-1 and HIV-2 by additional, more specific tests, contain antibodies against HIV-1 that cross-react with HIV-2 unless the results of other testing (such as virus culture or polymerase chain reaction) substantiate HIV-2 infection.

\*Specimens were considered positive by HIV-2 immunoblot if two of the following three bands were present: gp 140, gp 36, or p26. For HIV-1, if any two of these major bands: gp160/120, gp41, or p24 were present the sample was considered positive. Both HIV-1 and HIV-2 immunoblots were interpreted as negative if no bands were present and indeterminate if bands were present but the criteria for a positive interpretation were not met.

## Sensitivity Studies

### Reactivity in a Population from an HIV-2 Endemic Area

The ability of the GS HIV-2 EIA to detect antibodies to HIV-2 in specimens from an HIV-2 endemic area is shown in Table 4. The data include 338 serum samples obtained from women attending a family planning clinic in a West African country. All samples were tested in parallel with two licensed HIV-1 EIAs, an investigational immunoblot, and a research HIV-2 western blot; specimens positive by HIV-2 immunoblot were also tested by radioimmuno-precipitation (RIP).

**Table 4: Detection of Antibodies to HIV-2 in Women from a Family Planning Clinic from an Endemic Area**

GS HIV-2 EIA Reactivity	Reactivity by HIV-2 immunoblot and/or RIP		
	Positive	Indeterminate	Negative
Repeatedly Reactive	2	0	1
Negative	0	1	334

In this study, 99.1% of the specimens from a West African population that were tested were initially non-reactive, 0.9% were initially reactive and 0.9% were repeatedly reactive. The three

repeatedly reactive specimens were also tested with two different licensed HIV-1 EIAs. One specimen was non-reactive in both HIV-1 EIAs; one specimen was reactive in both HIV-1 EIAs and the third specimen was non-reactive in 1 licensed HIV-1 EIA, but reactive with the second HIV-1 EIA.

All specimens were also tested by HIV-1 and HIV-2 immunoblot, using the criteria described. Two of the three repeatedly reactive specimens were positive for antibodies for HIV-2 by immunoblot. One of these two specimens was indeterminate by HIV-1 immunoblot, the other was negative by HIV-1 immunoblot. The third GS HIV-2 EIA repeatedly reactive specimen was negative by both HIV-1 and HIV-2 immunoblot. The two HIV-2 immunoblot-positive specimens were also positive (displayed reactivity to gp160/140) by RIP. None of the remaining 335 samples was positive by HIV-1 or HIV-2 immunoblot. The two HIV-2 immunoblot-positive specimens included one which was positive in one of the two HIV-1 EIAs and one which was negative in both HIV-1 EIAs. These results demonstrate the ability of the GS HIV-2 EIA to detect samples positive for antibodies to HIV-2 in population-based screening, including samples that might be missed by screening with licensed HIV-1 EIAs.

### **Reactivity with Specimens from HIV-2 Seropositive Individuals**

In-house studies were performed on a total of 115 specimens from 115 individuals who had been tested for HIV-2 by radioimmunoprecipitation (RIP) and/or immunoblot, or who were regarded as likely to be HIV-2 seropositive because of risk factors, clinical symptoms, geographic location or because of questionable (atypical) HIV-1 results. The specimens from the latter group of individuals were designated as "Suspected HIV-2 Positive." All 115 specimens were correctly classified as repeatedly reactive. The results are shown in Table 5.

**Table 5: Reactivity of HIV-2 Seropositive Specimens in Several HIV-1 and HIV-2 Assays**

Clinical Diagnosis	EIA Results	GS HIV-2 EIA	HIV-2 RIP or immunoblot				HIV-1 POS	HIV-1 RIP or immunoblot	
			POS	IND	NEG	EIA		IND	NEG
AIDS (N=22)	Reactive	22	22	0	0	17	4	13	0
	Non-reactive	0	0	0	0	5	0	5	0
ARC (N=4)	Reactive	4	4	0	0	4	0	4	0
	Non-reactive	0	0	0	0	0	0	0	0
Other Medical Conditions <sup>a</sup> (N=7)	Reactive	7	7	0	0	2	1	1	0
	Non-reactive	0	0	0	0	5	0	5	0
Known Positive (N=70)	Reactive	70	70	0	0	62	18	44	0
	Non-reactive	0	0	0	0	8	1	5	2
Suspected HIV-2 Positive <sup>b</sup> (N=12)	Reactive	12	12	0	0	9	2	7	0
	Non-reactive	0	0	0	0	3	1	2	0
Total (N=115 <sup>c</sup> )	Reactive	115	115	0	0	94	25	69	0
	Non-reactive	0	0	0	0	21	2	17	2

<sup>a</sup> Other medical conditions included two individuals with tuberculosis, two with weight loss and lymphadenopathy, one with cirrhosis, one with neurological symptoms and one with lymphadenopathy.

<sup>b</sup> Specimens were classified as "Suspect HIV-2 Positive" based on risk factors, clinical symptoms, geographic location or because of questionable (atypical) HIV-1 results.

<sup>c</sup> HIV-2 was isolated from 14 of these 115 individuals. The 14 individuals included eleven patients with ARC/AIDS, one individual with other medical conditions, and two asymptomatic people (one was known to be positive for antibody to HIV-2, and one was suspected as being positive for antibody to HIV-2).

## Reactivity with HIV-1 Western Blot Indeterminate Specimens

In a study performed in the United States, repository specimens that had been classified as indeterminate by HIV-1 Western blot were screened for the presence of antibodies to HIV-2. Eleven specimens were found to be repeatedly reactive by the GS HIV-2 EIA. Seven of these were subsequently confirmed positive for antibodies to HIV-2 by immunoblot. Results are shown in Table 6 below. This study indicates that specimens which give indeterminate results on HIV-1 immunoblot (especially those that react with *gag* and *pol* only) should be investigated for the presence of antibodies to HIV-2.<sup>22</sup>

**Table 6: HIV-1 Western Blot Patterns of Indeterminate Samples Tested for Antibody to HIV-2**

HIV-1 Western Blot Banding Pattern	No. of Samples Tested	Reactivity in GS HIV-2 EIA		No. Positive by HIV-2 Immunoblot and RIP
		Reactive	Non-Reactive	
Gag only (p18, p24, and/or p55)	248	0	248	0
Polymerase only (p31, p51, or p66)	18	0	18	0
Gag plus Polymerase	60	9	51	6 <sup>a</sup>
Other <sup>b</sup>	31	2	29	1 <sup>c</sup>
Total	357	11	346	7 <sup>d</sup>

<sup>a</sup> All six specimens reacted with p24, p31, and p55. Some additional bands were present in several specimens; no definitive glycoprotein reactivity was observed with any of these six specimens.

<sup>b</sup> This group included 6 specimens with env only (gp41 or gp120/160), four with gag plus env, 18 with gag, pol and env and three that were negative by HIV-1 immunoblot.

<sup>c</sup> This specimen exhibited reactivity with gag and pol, and gave an atypical band result with env by HIV-1 immunoblot.

<sup>d</sup> The seven samples confirmed as positive for antibody to HIV-2 were taken from 5 individuals.

### Estimated Specificity

Specificity of the GS HIV-2 EIA was estimated from the results of screening tests in U.S. blood and plasma donors. Specificity was estimated by the following formula:

$$\frac{(\# \text{ normal donor specimens} - \# \text{ repeatedly reactive specimens})}{\# \text{ normal donor specimens}} \times 100$$

(# normal donor specimens - # repeatedly reactive specimens confirmed positive for antibodies to HIV-1)

A total of 8,023 donor specimens were tested; 21 of these specimens were repeatedly reactive by the GS HIV-2 EIA. Of the repeatedly reactive specimens, six were confirmed to be positive for antibodies to HIV-1. Thus the GS HIV-2 EIA has an estimated specificity of 99.8% (95% confidence interval: 99.7-99.9%).

### Estimated Sensitivity

Determining the sensitivity of the test for detection of antibodies to HIV-2 was limited by the difficulty in obtaining adequate numbers of samples from individuals who were infected with the virus and for whom previous serological test results were unknown.

Based on in-house studies showing a positive GS HIV-2 EIA in 22 of 22 AIDS patients with a positive Western blot for antibodies to HIV-2, the sensitivity is estimated to be 100% (95% confidence interval: 85-100%). This study included 18 cases with absence of antibody to HIV-1 envelope proteins. Similarly, detection was shown in 100% (14 of 14) of patients with a positive virus culture for HIV-2 (9 AIDS, 2 ARC, 1 neurologic disease, 2 asymptomatic).

Overall, the GS HIV-2 EIA was positive for 115 of 115 samples from several areas outside the U.S., investigated on the basis of suspected antibodies to HIV-2 and in which HIV-2 antibodies were confirmed by Western blot, including 88 cases negative for antibodies to HIV-1 envelope proteins.

Evidence of clinical sensitivity was obtained from prospective testing in an endemic area. The results showed detection by GS HIV-2 EIA of two samples with a positive HIV-2 Western blot and RIPA, one of which was not detected by either of two licensed HIV-1 EIAs.

## 14-BIBLIOGRAPHY

1. Barre-Sinoussi F, Chermann JC, Rey F, et al: Isolation of T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). **Science** 220:868-871, 1983.
2. Gallo RC, Salahuddin SZ, Popovic M, et al: Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. **Science** 224:500-503, 1984.
3. Coffin J, Haase A, Levy J, et al: What to call the AIDS virus? **Nature** 321:10, 1986.
4. Clavel F, Guetard D, Brun-Vezinet F: Isolation of a new human retrovirus from West African patients with AIDS. **Science** 233:343-346, 1986.
5. Horsburgh C, Holmberg S: The global distribution of human immunodeficiency virus type 2 (HIV-2) infection. **Transfusion** 28:192-195, 1988.
6. Hoff R, Weiblen B, Schwerzler B, et al: Specific antibodies to HIV-2 detected in an anonymous newborn blood specimen from Massa-

- chusetts. **Fourth Consensus Conference on Testing for Human Retroviruses**, March 1989.
7. Ayanian J, Maguire J, Marlink R, et al: HIV-2 infection in the United States. **New Engl J Med** 320:1422-1423, 1989.
  8. Centers for Disease Control: Update: HIV-2 infection among blood and plasma donors - United States, June 1992 - June 1995. **MMWR** 44:603-606, 1995.
  9. O'Brien TR, George JR, Holmberg SD: Human immunodeficiency virus type 2 infection in the United States. **JAMA** 267:2775-2779, 1992.
  10. Brun-Vezinet F, Katlama C, Roulot D, et al: Lymphadenopathy associated virus type 2 in AIDS and AIDS-related complex. **Lancet** i:128-132, 1987.
  11. Quinn T, Zacarias F, St. John R: AIDS in the Americas: an emerging public health crises. **New Engl J Med** 320:1105-1007, 1989.
  12. Guyader M, Emerman M, Sonigo M, et al: Genome organization and transactivation of the human immunodeficiency virus type 2. **Nature** 326-662, 1987.
  13. Cabrian K, Shriver K, Goldstein L, et al: Human Immunodeficiency Virus Type 2: a review. **J Clinical Immunoassay** 11:107-114, 1988.
  14. George R, Rayfield M, Schochetman G, et al: Sensitivity of U.S. FDA licensed HIV-1 enzyme immunoassays for detection of HIV-2 antibodies. **Abstracts: 1989 V International Conference on AIDS**, section B, page 306.
  15. Bos ES, van der Doelen AA, van Rooy N, Schuur AHWM: 3, 3', 5, 5' - tetramethylbenzidine as an ames test negative chromogen for horseradish peroxidase in enzyme immunoassay. **J Immunoassay** 2:187-204, 1981.
  16. Garner RC, Walpole AL, Rose FL: Testing of some benzidine analogues for microsomal activation to bacterial mutagens. **Cancer Letters** 1:39-42, 1975.
  17. Resnick L, Veren K, Salahuddin SZ, et al: Stability and inactivation of HTLV-III/LAV under clinical and laboratory environments. **JAMA** 255:1887-1891, 1986.
  18. Sarngadharan MG, Markham PD: The role of human T-lymphotropic retroviruses in leukemia and AIDS, in Wormser GP (ed): **AIDS and Other Manifestations of HIV Infection**. New Jersey, Noyes Publications, 1987, pp. 218-220.

19. Bond WW, Favero MS, Petersen NJ, et al: Inactivation of hepatitis B virus by intermediate-to-high level disinfectant chemicals. **J Clin Micro** 18:535-538, 1983.
20. Schulster LM, Hollinger FB, Dreesman GR, Melnick JL: Immunological and biophysical alteration of hepatitis B virus antigens by sodium hypochlorite disinfection. **Appl Environ Microbiol** 42:762-7, 1981.
21. Centers for Disease Control and Prevention: 1993 Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS Among Adolescents and Adults. **MMWR** 41 (No. RR-17):1-19, 1992.
22. Report and Recommendations, Committee on HIV, Association of State and Territorial Public Health Laboratory Directors, **Fourth Consensus Conference on Testing for Human Retroviruses**, March 1989.

For Reference Use Only

**BIO-RAD**

**Bio-Rad  
Laboratories**

---

**Redmond, WA 98052, U.S.A.**

**U.S. License No. 1109**

**For Customer Orders or Technical Service call:  
1-800-2BIORAD (1-800-224-6723)**

---

Revised: June 2007  
506103