HUMAN IMMUNODEFICIENCY VIRUS TYPES 1 AND 2 (Synthetic Peptide)

Genetic Systems™ HIV-1 / HIV-2 Peptide EIA

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

For in vitro diagnostic use

32551 • 480 Tests
32542 • 960 Tests
32543 • 4800 Tests

Manufactured for Sanofi Diagnostics Pasteur, Inc. by
Genetic Systems Corporation
U.S. License No. 978

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sanofi DIAGNOSTICS Pasteur

NAME AND INTENDED USE

Genetic Systems™ HIV-1/HIV-2 Peptide EIA is the Genetic Systems Corporation qualitative enzyme immunoassay for the detection of antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) and/or Human Immunodeficiency Virus Type 2 (HIV-2) in human serum and plasma, and also in cadaveric serum specimens. The HIV-1/HIV-2 Peptide EIA is intended for screening blood and blood products intended for transfusion or for further manufacture into plasma products.

SUMMARY AND EXPLANATION

The acquired immunodeficiency syndrome (AIDS) is caused by viruses transmitted by sexual contact, exposure to blood (including sharing contaminated needles and syringes) or certain blood products, or transmitted from an infected mother to her fetus or child during the perinatal period.[1] Additionally, transmission of HIV and other infectious diseases can occur through tissue transplantation.[2] Human Immunodeficiency Virus Type 1 (HIV-1) has been isolated from patients with AIDS and AIDS-related complex (ARC), and the virus has been characterized extensively.[3-7] 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.[8,9] 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.[10] In the United States, there have been more than 50 cases of infection with HIV-2 reported.[11-16]

This second immunodeficiency virus is similar to, but distinct from, HIV-1. Both viruses have similar morphology and lymphotropism,[17] and the modes of transmission appear to be identical.[10,18] In addition, the HIV-1 and HIV-2 genomes exhibit about 60% homology in conserved genes such as *gag* and *pol*, and 35-45% homology in the envelope genes.[19] 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.[20]

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.[21] The Genetic Systems™ HIV-1/HIV-2 Peptide EIA is comprised of four highly conserved, immunodominant peptide sequences representing HIV-1 and HIV-2.[22-28] The peptide sequences were derived from three different, prevalent virus strains. The Genetic Systems™ HIV-1/HIV-2 Peptide EIA was developed to improve both the sensitivity and specificity of the detection of antibodies to HIV-1 and/or HIV-2 for blood screening and to aid in diagnosis of HIV infection.

Any specimen that reacts in an initial test (is initially reactive) with the Genetic Systems™ HIV-1/HIV-2 Peptide EIA must be retested in duplicate with the Genetic

Systems™ HIV-1/HIV-2 Peptide 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, immunofluorescence, or radioimmunoprecipitation must be performed to verify presence of antibodies to HIV. Recommendations for appropriate use of such additional tests may be issued periodically by the United States Public Health Service.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The Genetic Systems™ HIV-1/HIV-2 Peptide EIA is manufactured using synthetic peptides derived from highly conserved, immunodominant regions of the *env* (envelope) and *pol* (polymerase) gene products for HIV-1 and HIV-2. The microwells are coated with a mixture of four peptides: *env* and *pol* sequences for both HIV-1 and HIV-2.

During the assay, specimens are evaluated for the presence of HIV-1 and HIV-2 antibodies by interaction with the adsorbed peptides in the wells. Specimens to be tested are diluted in Specimen Diluent and added to each well, and the plates are incubated and washed. If antibodies to either HIV-1 or HIV-2 are present, they bind to the adsorbed antigen and are not removed by washing. The Working Conjugate Solution, 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, Working Chromogen Solution 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, which results in a color change to yellow. The optical absorbance of controls and specimens is determined with a spectrophotometer with wavelength set at 450 nm.

REAGENTS

Genetic Systems™ HIV-1/HIV-2 Peptide EIA Product Description
Product No: 32551 (480 Tests), 32542 (960 Tests), 32543 (4800 Tests)

Component	Contents	Preparation
R1 • HIV-1/HIV-2 Peptide Coated Microwell Plates, 5, 10, or 50	•Microwell strips with adsorbed HIV-1 and HIV-2 peptides. •0.1% Proclin 150™ preservative	Use as supplied.
C0 •HIV Negative Control, 1, 1, or 5 vial(s) (1.8ml)	•Human serum •Non-reactive for HBsAg and antibodies to HIV-1, HIV-2, HTLV-I/II, and HCV. •0.1% Sodium azide •0.01% Thimerosal	Dilute in Specimen Diluent as described.
C1 •HIV-1 Positive Control, 1, 1, or 5 vial(s) (1.6ml)	•Human serum containing HIV-1 immunoglobulin •Specific for HIV-1 by EIA •Non-reactive for HBsAg •Non-reactive for antibody to HTLV-I/II andHCV •0.1% Sodium azide •0.01% Thimerosal	Dilute in Specimen Diluent as described.
C2 •HIV-2 Positive Control, 1, 1, or 5 vial(s) (1.6ml)	•Human serum containing HIV-2 immunoglobulin •Specific for HIV-2 by EIA •Non-reactive for HBsAg •Non-reactive for antibody to HTLV-I/II and HCV •0.1% Sodium azide •0.01% Thimerosal	Dilute in Specimen Diluent as described.
R2 • HIV-1/HIV-2 Peptide EIA Specimen Diluent 1, 2, or 10 bottle(s) (120ml)	•Normal goat serum •0.1% Proclin 300™ preservative	Diluent for specimens and controls. Ready to use as supplied.
R3 • HIV-1/HIV-2 Peptide EIA Conjugate Concentrate, 1, 1, or 5 vial(s) (1.8ml)	•Goat anti-human IgM and IgG horseradish peroxidase conjugated solution •0.01% Thimerosal	Dilute in Conjugate Diluent as described.
R4 • HIV-1/HIV-2 Peptide EIA Conjugate Diluent, 1, 1, or 5 bottle(s) (120ml)	•Normal goat serum •Normal bovine serum •0.1% Proclin 150™ preservative	Diluent for Conjugate Concentrate. Ready to use as supplied.
R5 • EIA Wash Solution Concentrate(30X), 2, 2, or 10 bottle(s) (120ml)	•Sodium chloride •Tween 20	Dilute to working concentration with deionized or distilled water.
R6 • EIA Chromogen Reagent, 1, 1, or 5 vial(s) (1.5ml)	•Tetramethylbenzidine (TMB)** •Dimethylsulfoxide (DMSO)	Dilute in EIA Chromogen Diluent as described.
R7 • EIA Chromogen Diluent, 1, 1, or 5 bottle(s) (120ml)	•Hydrogen peroxide •Citric acid •Dimethylsulfoxide (DMSO)	Ready to use as supplied.
R8 • EIA Stopping Reagent, 1, 1, or 5 bottle(s) (120ml)	•1N H_2SO_4	Ready to use as supplied.
• Plate Sealers	•Clear plastic sealers	Ready to use as supplied.

**Note: Tetramethylbenzidine is a non-carcinogenic and non-mutagenic chromogen for peroxidase.[29,30]

Store the kit at 2-8°C. Bring all reagents except HIV-1/HIV-2 Peptide EIA Conjugate Concentrate to room temperature (15-30°C) before use. Return all reagents to 2-8°C after use. Return unused strips/plates to pouch and reseal. Do not remove desiccant. **Strips should be used within three months of opening and resealing the pouch.** Store strips/plates at 2-8°C.

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. The HIV-1 and HIV-2 Positive Controls are heat-treated to inactivate viruses. However, handle all the reagents as though capable of transmitting infection. All tests should be conducted using the precautions recommended for bloodborne pathogens, as defined by OSHA regulations.

2. Do not pipette by mouth.

3. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.

4. Wear protective clothing and disposable gloves while handling the kit reagents. Wash hands thoroughly after performing the test.

5. Handle Chromogen Reagent with care, since DMSO is readily absorbed through the skin.

6. The Stopping Reagent is an acid. Wipe up spills immediately and flush the area with water. If the Stopping Reagent contacts the skin or eyes, flush with copious amounts of water and seek medical attention.

7. BIOLOGICAL SPILLS: Spills not containing acid should be wiped thoroughly with an effective disinfectant. Disinfectants that can be used include (but are not limited to) a solution of 10% bleach (0.5% solution of sodium hypochlorite), 70% ethanol, or 0.5% Wescodyne™.[31-33] Spills containing acid should be wiped dry. The area of the spill should be wiped with one of the chemical disinfectants. Materials used to wipe up spills should be disposed of as biohazardous waste. NOTE: DO NOT PLACE SOLUTIONS CONTAINING BLEACH IN THE AUTOCLAVE.

8. *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.[33,34]

9. Sodium azide is included as a preservative in the positive and negative controls. Sodium azide has been reported to form lead or copper azides in laboratory plumbing. These azides are explosive. To prevent azide build-up, flush plumbing with a large volume of water if solutions containing azide are disposed of in the sink after biological inactivation.

PRECAUTIONS FOR USERS

1. Do not use the kit or any kit reagents beyond the stated expiration date.
2. The only reagents that may be used with different lots of the HIV-1/HIV-2 Peptide EIA are the Chromogen Reagent, Chromogen Diluent, Wash Solution Concentrate and Stopping Reagent. Do not use any other reagents from different lots.
3. Do not use the Chromogen Diluent for the EIA Buffered Substrate in other Genetic Systems tests.
4. Exercise care in opening and removing aliquots from vials to avoid microbial contamination of the reagents.
5. Use a clean container for Working Conjugate Solution. Exposure of Conjugate Diluent or Concentrate to sodium azide or serum will inactivate Conjugate Solution. Avoid prolonged exposure to light.
6. Avoid exposing Chromogen Reagent or the Working Chromogen Solution to strong light during storage or incubation. Do not allow the chromogen solutions to come into contact with an oxidizing agent.
7. Use clean **polypropylene** containers **(DO NOT USE POLYSTYRENE CONTAINERS)** to prepare and store the Working Chromogen Solution. If glassware must be used, pre-rinse thoroughly with 1N sulfuric or hydrochloric acid followed by at least three washes of deionized water. Be sure that no acid residue remains on the glassware. If polypropylene containers are to be reused, they should be cleaned in accordance with a cleaning process validated by the testing facility.
8. Avoid contact of Stopping Reagent with any oxidizing agent. Do not allow Stopping Reagent to come into contact with metals.
9. Bring all reagents except the Conjugate Concentrate to room temperature before use.
10. For the manual pipetting of controls and specimens, use individual pipette tips to eliminate carryover of samples.
11. Handle negative and positive controls in the same manner as patient specimens.
12. If a specimen is inadvertently not added to a well, the assay result will read nonreactive.
13. Inadequate adherence to package insert instructions may result in erroneous or invalid results.
14. The Genetic Systems™ HIV-1/HIV-2 Peptide EIA performance is highly dependent upon incubation times and temperatures. Temperatures outside of the validated ranges may result in invalid assays. Incubation temperatures

should be carefully monitored using calibrated thermometers, or equivalent.

15. Use only adequately calibrated equipment with this assay.

16. Use of dedicated equipment is recommended if equipment performance validations have not precluded the possibility of cross-contamination.

17. Components of this kit meet FDA potency requirements.

REAGENT PREPARATION AND STORAGE

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 adding 10 µl of Conjugate Concentrate to 1 ml of Conjugate Diluent in a clean container. Note Concentrate lot number, date and time of preparation, and time of expiration of the Working Conjugate Solution. Mix Working Solution prior to use. Working Solution is stable for 8 hours at room temperature.

Return Conjugate Concentrate to refrigerator immediately after use. To avoid contamination of Conjugate, wear clean gloves and do not touch tips of pipettes. Store Working Conjugate Solution at room temperature until use. Avoid prolonged exposure to light.

Do not add all the Concentrate to Diluent. Prepare only the amount of reagent to be used within 8 hours, ensuring that the volume of diluted reagent will be adequate for the entire plate(s). Use the following table as a guide:

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

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 (µl)	120	240	360	480	600	720	840	960	1080	1200
Amount of Conjugate Diluent (ml)	12	24	36	48	60	72	84	96	108	120

Working Chromogen Solution

Bring Chromogen Reagent and Chromogen Diluent to room temperature. Invert the Chromogen Reagent and Chromogen Diluent to mix before using. Prepare a 1:101 dilution for each strip to be tested by adding 10 µl of Chromogen Reagent to 1 ml of Chromogen Diluent in a clean **polypropylene** container. **(DO NOT USE A POLYSTYRENE CONTAINER).** Note Chromogen Reagent lot number, date and time of preparation, and time of expiration of the Working Chromogen Solution. Mix Working Solution gently when combined. Working Chromogen Solution should be kept in the dark at room temperature 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. If solution remains crystalline after warming, do not 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.

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 plate(s). Extra Chromogen Reagent is provided. Use the following table as a guide:

Preparation of 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 Chromogen Reagent (µl)	10	20	30	40	50	60	70	80	90	100	110	120
Amount of Chromogen Diluent (ml)	1	2	3	4	5	6	7	8	9	10	11	12

* Complete Plate

Preparation of Working Chromogen Solution by Plate

Number of Complete Plates to be used	1	2	3	4	5	6	7	8	9	10
Amount of Chromogen Reagent (µl)	120	240	360	480	600	720	840	960	1080	1200
Amount of Chromogen Diluent (ml)	12	24	36	48	60	72	84	96	108	120

Working Wash Solution

Prepare Working Wash Solution as needed by adding one part Wash Solution Concentrate (30X) to 29 parts of water (e.g., 120 ml of Wash Solution to 3480 ml of water). Use deionized or distilled water. Clinical laboratory reagent water Type I or Type II is acceptable. The Working Wash Solution can be stored at room temperature for four weeks. Note lot number, date prepared, and expiration date. Discard if no foaming is evident in the Working Wash Solution. Prepare a sufficient quantity of Working Wash Solution to complete a full plate run.

SPECIMEN COLLECTION, PREPARATION AND STORAGE

Serum or Plasma

Serum, plasma, or cadaveric serum specimens may be used in the test. The following anticoagulants have all been evaluated and found to be acceptable: EDTA, 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.

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 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.

GENETIC SYSTEMS™ HIV-1/HIV-2 PEPTIDE EIA PROCEDURE

Materials Required

See Reagents Section on page 4.

Materials Required But Not Provided

1. Precision pipettes to deliver 0-20 µl, 20-200 µl, 1 ml, 5 ml, and 10 ml (accurate within ± 10%) or automated pipettor-dilutor; appropriately-sized graduated cylinders.
2. Pipette tips.
3. **Dry-heat incubator capable of maintaining 37 ±1°C.**
4. Calibrated thermometer.
5. Genetic Systems microwell plate or strip washer or an equivalent. The washer must be capable of dispensing at least 350 µl per well and cycling 5 times.
6. Genetic Systems microwell plate or strip reader or an equivalent. The spectrophotometer should have the following specifications at wavelength 450 nm:

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

   The instrument should contain a reference filter for reading at 615 to 630 nm. 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.
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™ (West Chemical Products, Inc.).
8. Paper towels or absorbent pads for blotting.
9. Null Strips, for testing partial plates.
10. Clean **polypropylene** container for preparation of Working Chromogen Solution. **(DO NOT USE POLYSTYRENE).** Clean container for preparation of Working Conjugate Solution.
11. Deionized or distilled water. Clinical laboratory reagent water Type I or Type II is acceptable.[35] Store the water in nonmetallic containers.
12. Gloves.
13. Laboratory timer.

14. EIA reagent reservoirs (optional).

Preliminary Statements

1. Each run of this assay must proceed to completion without interruption after it has been started.
2. Positive and negative controls must be run on each plate. The cutoff value for specimens is determined by the controls on each individual plate.
3. The number of controls to be included in each run of this assay are two HIV-1 Positive Controls, two HIV-2 Positive Controls, and three Negative Controls.
4. Do not splash controls, specimens, or reagents between microwells of the plate.
5. Cover plates for each incubation step using plate sealers provided or other appropriate means to minimize evaporation.
6. Avoid exposure of the plates to light during the final incubation step (following the addition of the Working Chromogen Solution).
7. Adhere to the recommended time constraints for the use of the Working Chromogen Solution (8 hours), Working Conjugate Solution (8 hours), and Working Wash Solution (4 weeks).
8. Avoid the formation of air bubbles in each microwell.

EIA Procedure

Note: Serum, plasma, or cadaveric specimens are diluted 1:10 in HIV-1/HIV-2 Peptide EIA Specimen Diluent prior to testing.

1. Perform equipment maintenance and calibration, where necessary, as required by the manufacturer.
2. **Bring all of the reagents except the HIV-1/HIV-2 Peptide EIA Conjugate Concentrate to room temperature** before beginning the assay procedure.
3. Prepare Working Wash Solution, Working Conjugate Solution, and Working Chromogen Solution. See Reagent Preparation Section. Mix gently prior to use.
4. Remove any strips from the microwell plate(s) not needed for the assay run and replace with Null Strips, if necessary.
5. If sample identity is not maintained by an automatic procedure, label or identify the individual wells for each specimen or control on a data sheet.
6. **Dilute specimens and controls 1:10 in the Specimen Diluent.** (For example, dilute 15 µl of specimen in 135 µl of Specimen Diluent.) When pipetting manually, use a separate, disposable pipette tip for each specimen.

Two separate dilutions of both HIV-1 and HIV-2 Positive Controls and three separate dilutions of Negative Control should be assayed with each plate or partial plate of specimens. Mix each diluted specimen and control thoroughly. Mix gently to avoid foaming of the diluent. All controls and specimens must be subjected to the same process.

7. **Add 100 µl of the diluted specimen or control to the appropriate well OR if doing in-well dilutions, combine 10µl of specimen or control with 90µl of Specimen Diluent.**

8. Cover the microwell plate with a plate sealer or use other means to minimize evaporation and **incubate the plate for 30 to 33 minutes at 37 ± 1°C.**

9. 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 microwell plate or strip a minimum of five times** with the Wash Solution (at least 350 µl/well/wash), or as otherwise validated. Aspirate the Wash Solution after each wash. After the last wash, aspirate the liquid completely or blot the inverted plate on clean, absorbent paper towels, if necessary. Note: Grasp the plate holder firmly at the center of the long sides before inverting to blot.

10. **Add 100 µl of Working Conjugate Solution to each well.**

11. Cover the microwell plate with a fresh plate sealer or use other means to minimize evaporation and **incubate the plate for 30 to 33 minutes at 37 ± 1°C.**

12. 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 microwell plate or strip a minimum of five times** with the Wash Solution (at least 350 µl/well/wash), or as otherwise validated. Aspirate the Wash Solution after each wash. After the last wash, aspirate the liquid completely or 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.

13. **Add 100 µl of the Working Chromogen Solution per well.** Cover the microwell plate with a fresh plate sealer or use other means to minimize evaporation. **Incubate plates in the dark for 30 to 33 minutes at room temperature (15 to 30°C).** (e.g., cover the plates with opaque plastic or place in a drawer.)

14. Carefully remove the plate cover and **add 100 µl of Stopping Reagent 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 Reagent, using the 450 nm filter with 615 nm to 630 nm filter as the reference. (Blank on air.) 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.[33,34]

QUALITY CONTROL

Determine the mean absorbance for the Negative and Positive Controls by dividing the sum of their absorbance values by the number of acceptable controls.

Mean Negative Control absorbance value (NCx)

The individual negative control absorbance values must be greater than or equal to 0.020 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.

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

Negative Control Sample Number	Absorbance
1	0.095
2	0.110
3	0.102
	0.307

$$\frac{\text{Total absorbance}}{3} = \frac{0.307}{3} = 0.102 \text{ (NCx)}$$

Mean HIV-1 Positive Control absorbance value (HIV-1 PCx)

Determine the mean of the HIV-1 Positive Control as shown in the example below.

HIV-1 Positive Control Sample Number	Absorbance
1	1.435
2	1.501
	2.936

$$\frac{\text{Total absorbance}}{2} = \frac{2.936}{2} = 1.468 \text{ (HIV-1 PCx)}$$

The HIV-1 PCx must be greater than or equal to 0.900 AU, and each Positive Control absorbance value must be within the reproducibility range of 0.65 to 1.35 times the PCx. No Positive Control absorbance value may be discarded.

Both of the HIV-1 Positive Control absorbance values above are within the reproducibility range of 0.65 to 1.35 times the PCx as shown by the following calculation:

$$0.65 \times \text{(HIV-1 PCx)} = 0.65 \times 1.468 = 0.954$$
$$1.35 \times \text{(HIV-1 PCx)} = 1.35 \times 1.468 = 1.982$$

Therefore, the acceptable range is 0.954 to 1.982.

Mean HIV-2 Positive Control absorbance value (HIV-2 PCx)

Determine the mean of the HIV-2 Positive Control as shown in the example below.

HIV-2 Positive Control

Sample Number	Absorbance
1	1.078
2	1.123
	2.201

$$\frac{\text{Total Absorbance}}{2} = \frac{2.201}{2} = 1.101 \text{ (HIV-2 PCx)}$$

The HIV-2 PCx must be greater than or equal to 0.700 AU, and each Positive Control absorbance value must be within the reproducibility range of 0.65 to 1.35 times the PCx. No Positive Control absorbance value may be discarded.

Both of the HIV-2 Positive Control absorbance values above are within the reproducibility range of 0.65 to 1.35 times the PCx as shown by the following calculation:

$$0.65 \times \text{(HIV-2 PCx)} = 0.65 \times 1.101 = 0.716$$
$$1.35 \times \text{(HIV-2 PCx)} = 1.35 \times 1.101 = 1.486$$

Therefore, the acceptable range is 0.716 to 1.486.

Cutoff Value

Determine the cutoff value by adding 0.240 to the NCx, as shown in the example below:

$$\text{NCx} = 0.102$$
$$\text{Cutoff Value} = 0.102 + 0.240 = 0.342$$

Validity Criteria

A run is valid if the following criteria are met:

- The absorbance value of each Negative Control is greater than or equal to 0.020 AU and less than or equal to 0.140 AU. One Negative Control value may be discarded, and the mean of the Negative Controls (NCx) may be calculated from the two remaining values.
  If two or more Negative Controls are out of limit, the plate is invalid and must be repeated.
- The mean absorbance of the HIV-1 Positive Control is equal to or greater than 0.900 AU, and the individual absorbance values are within the reproducibility range of 0.65 to 1.35 times the HIV-1 Positive Control mean. No HIV-1 Positive Control values may be discarded.
  If the HIV-1 Positive Control values are out of the reproducibility range, or if the HIV-1 Positive Control mean is less than 0.900 AU, the plate is invalid and must be repeated.
- The mean absorbance of the HIV-2 Positive Control is equal to or greater than 0.700 AU, and the individual absorbance values are within the reproducibility range of 0.65 to 1.35 times the HIV-2 Positive Control mean. No HIV-2 Positive Control values may be discarded.
  If the HIV-2 Positive Control values are out of the reproducibility range, or if the HIV-2 Positive Control mean is less than 0.700 AU, the plate is invalid and must be repeated.

INTERPRETATION OF RESULTS

The presence or absence of antibodies to HIV-1 and/or HIV-2 is determined by relating the absorbance value of the specimen to the cutoff value. The cutoff value is determined by adding 0.240 to the mean absorbance value of the Negative Controls. An example of values obtained from an assay run and the interpretations are as follows:

Example:

Negative Control AU values	0.095 0.110 0.102	Negative Control mean	0.102 (valid)
HIV-1 Positive Control AU values	**1.435** **1.501**	HIV-1 Positive Control mean HIV-1 Positive Control acceptable range	1.468 (valid) 0.954 - 1.982
HIV-2 Positive Control AU values	**1.078** **1.123**	HIV-2 Positive Control mean HIV-2 Positive Control acceptable range	1.101 (valid) 0.716 - 1.486
Cutoff Value = 0.102 + 0.240 =	0.342		
Patient AU values	0.047 **1.910** **0.395** 0.095 **0.726** 0.100	Interpretation	Nonreactive **Reactive** **Reactive** Nonreactive **Reactive** Nonreactive

1. Specimens with absorbance values less than the cutoff value are considered non-reactive by the Genetic Systems™ HIV-1/HIV-2 Peptide EIA and may be considered negative for antibody to HIV-1 and 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 equal to or greater than the cutoff value are considered initially reactive by the Genetic Systems™ HIV-1/HIV-2 Peptide EIA and should be retested in duplicate before interpretation. When tube dilutions are used to mix the specimen with Specimen Diluent, prepare a new dilution of the specimen for retesting. If, after repeat testing, the absorbance of either or both duplicate specimens is greater than or equal to the cutoff value, the specimen is considered repeatedly reactive. Those specimens with values greater than the upper linearity limits of the reader should be reported as reactive.

4. Initially reactive specimens that do not react in either of the duplicate repeat tests are considered negative for antibodies to HIV-1 and HIV-2.

5. If the specimen is repeatedly reactive, the probability that antibodies to HIV-1 and/or HIV-2 are present is high, especially for specimens obtained from subjects at increased risk for HIV-1 and/or HIV-2 infection or for specimens with very high absorbance values. In most settings, it is appropriate to investigate repeatedly reactive specimens by additional, more specific or supplemental tests, such as Western blot or immunofluorescence.
   - Specimens that are repeatedly reactive by the Genetic Systems™ HIV-1/HIV-2 Peptide EIA and are found to be positive for antibodies to HIV-1 by additional, more specific or supplemental testing but negative or indeterminate for antibodies to HIV-2 are considered to be positive for antibodies to HIV-1.
   - Specimens that are repeatedly reactive by the Genetic Systems™ HIV-1/HIV-2 Peptide EIA and are found to be positive by additional, more specific or supplemental testing for antibodies to HIV-2 but negative or indeterminate for antibodies to HIV-1 are considered to be positive for antibodies to HIV-2.
   - Specimens that are repeatedly reactive by the Genetic Systems™ HIV-1/HIV-2 Peptide EIA and are found to be positive by additional, more specific or supplemental testing for both HIV-1 and HIV-2 antibodies may contain antibodies that cross-react with both virus types, or may be indicative of a dual infection with both HIV-1 and HIV-2.
   - The interpretation of results of specimens found to be repeatedly reactive by Genetic Systems™ HIV-1/HIV-2 Peptide EIA and negative or indeterminate on additional, more specific testing for antibodies to both HIV-1 and HIV-2 is unclear. Clarification may sometimes be obtained by testing another specimen taken three to six months later.

LIMITATIONS OF THE PROCEDURE

1. The Genetic Systems™ HIV-1/HIV-2 Peptide 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. Data regarding the interpretation were derived from testing serum, plasma, or cadaveric serum 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. The Genetic Systems™ HIV-1/HIV-2 Peptide EIA detects antibodies to HIV-1 and HIV-2 and thus is useful in screening blood and plasma donated for transfusion and further manufacture, in screening cadaveric serum for tissue donation, in evaluating patients with signs or symptoms of AIDS, and in establishing prior infection with HIV-1 or HIV-2. Clinical studies continue to clarify and refine the interpretation and medical significance of the presence of antibodies to HIV-1 or HIV-2.[10] 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, and these should be considered an important part of testing for antibody to HIV-1 and HIV-2 including confirmation of the test result on a freshly drawn sample.

3. AIDS and AIDS-related conditions are clinical syndromes and their diagnosis can only be established clinically.[36,37] 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. False 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.[10,38,39] However, in a prospective study, AIDS developed in 51% of homosexual men after 10 years of infection.[40]

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 Peptide EIA may be more likely to demonstrate the presence of antibodies to HIV-1 and/or HIV-2 by additional, more specific, or supplemental testing.[41] Borderline reactivity is more frequently nonspecific, especially in samples obtained from persons at low risk for infection with HIV-1 or HIV-2; however, the presence of antibodies to HIV-1 and/or HIV-2 in some of these specimens can be demonstrated by additional, more specific, or supplemental testing, or by testing a subsequent sample drawn at a later date (e.g. 3 to 6 months).[42]

9. It is generally recognized that detection and confirmation 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.[43]

10. An absorbance value of less than 0.000 AU may indicate a procedural or instrument error which should be evaulated. 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 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 Reagent solution by oxidizing agents (sodium hypochlorite, hydrogen peroxide, etc).
- contamination of the Stopping Reagent

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.

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 6 dilutions of an HIV-1 antibody-positive specimen, 6 dilutions of an HIV-2 antibody-positive specimen, and 2 seronegative specimens. The specimens were tested 6 times on 4 different days using 3 different test kit lots at each of 7 sites. The data were analyzed at Genetic Systems according to the National Committee for Clinical Laboratory Standards (NCCLS)[a and b]. The mean Absorbance Unit (AU), standard deviation (SD), and percent coefficient of variation (%CV) for each panel member are listed in Table 1 below.

Table 1: Reproducibility of the Genetic Systems™ HIV-1/HIV-2 Peptide EIA

Inter-assay Reproducibility					Intra-assay Reproducibility				
Specimen	N*	Mean AU	SD[a]	%CV	Specimen	N*	Mean AU	SD[b]	%CV
1	503	0.245	0.051	20.8%	1	503	0.245	0.021	8.6%
2	500	0.712	0.139	19.5%	2	500	0.712	0.046	6.5%
3	503	0.762	0.123	16.1%	3	503	0.762	0.055	7.2%
4	504	0.446	0.092	20.6%	4	504	0.446	0.031	7.0%
5	503	1.472	0.193	13.1%	5	503	1.472	0.079	5.4%
6	502	0.440	0.079	18.0%	6	502	0.440	0.035	8.0%
7	503	1.469	0.157	10.7%	7	503	1.469	0.069	4.7%
8	497	0.066	0.020	30.3%	8	497	0.066	0.009	13.6%
9	499	0.065	0.019	29.2%	9	499	0.065	0.008	12.3%
10	500	1.966	0.121	6.2%	10	500	1.966	0.050	2.5%
11	503	0.229	0.052	22.7%	11	503	0.229	0.017	7.4%
12	499	0.127	0.030	23.6%	12	499	0.127	0.009	7.1%
13	497	0.123	0.034	27.6%	13	497	0.123	0.011	8.9%
14	502	1.917	0.159	8.3%	14	502	1.917	0.062	3.2%

*Outliers not included in statistical calculations
[a]NCCLS Vol. 12 No.4, p.33 Eq's 12 and 13
[b]NCCLS Vol. 12 No.4, p.32 Eq 11

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 on specimens from random U.S. and Canadian blood donors and specimens from individuals with medical conditions unrelated to HIV-1 or HIV-2 infection are summarized in Table 2. The data include 19,968 serum and plasma samples obtained from donors at six geographically distinct locations, and 356 specimens from individuals with various medical conditions.

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

	Results Obtained with HIV-1/HIV-2 Peptide EIA				Repeatedly Reactive Specimens	
Group	Number Tested	Non-Reactive	Initially Reactive	Repeatedly Reactive	HIV-2 EIA Repeatedly Reactive	Pos. by HIV-1 Immunoblot alone
Random Donors, Site 1[a]	2,000 (100.00%)	1,998 (99.90%)	2 (0.10%)	1 (0.05%)	0	0
Random Donors, Site 2[a]	2,250 (100.00%)	2,244 (99.73%)	6 (0.27%)	2 (0.09%)	0	0
Random Donors, Site 3[a]	2,016 (100.00%)	2,012 (99.80%)	4 (0.20%)	3 (0.15%)	0	0
Random Donors, Site 4[a]	2,000 (100.00%)	1,998 (99.90%)	2 (0.10%)	2 (0.10%)	0	0
Random Donors, Site 5[a]	4,545 (100.00%)	4,535 (99.78%)	10 (0.22%)	10 (0.22%)	0	0
Random Donors, Site 6[b]	7,157 (100.00%)	7,148 (99.87%)	9 (0.13%)	7 (0.10%)	0	0
TOTAL:	**19,968 (100.00%)**	**19,935 (99.83%)**	**33 (0.17%)**	**25 (0.13%)**	**0**	**0**
Bacterial/Parasitic Diseases[c]	43 (100.00%)	42 (97.67%)	1 (2.33%)	0 (0.00%)	NA	NA
Autoimmune Diseases[d]	76 (100.00%)	73 (96.05%)	3 (3.95%)	3 (3.95%)	0	0
Other Viral Diseases[e]	161 (100.00%)	159 (98.76%)	2 (1.24%)	1 (0.62%)	0	0
Malignancies[f]	23 (100.00%)	23 (100.00%)	0 (0.00%)	0 (0.00%)	NA	NA
Other Specimens[g]	53 (100.00%)	53 (100.00%)	0 (0.00%)	0 (0.00%)	NA	NA
TOTAL:	**356 (100.00%)**	**350 (98.31%)**	**6 (1.69%)**	**4 (1.12%)**	**0**	**0**

a Serum was tested at sites 2 and 4; plasma was tested at sites 1, 3, and 5.
b Serum - 1,959; Plasma - 5,198
c 23 toxoplasmosis, 20 RPR+
d 15 Rheumatoid factor positive, 6 Rheumatoid arthritis; 1 Rheumatoid arthritis/Hepatitis; 2 Sjogrens; 1 SLE/Sepsis; 1 SLE/Staph. aureus; 20 ANA+; 18 Elevated IgG; 12 Elevated IgM
e 20 HBsAg+; 7 anti-HTLV-I+; 5 anti-HTLV-II+; 8 anti-HTLV-II+; 20 Anti-CMV+; 10 Anti-EBV+; 11 Anti-EBVCA+; 10 Anti-HAV IgM+; 12 Anti-HAV Total+; 22 Anti-HCV+; 20 Anti-HSV+; 16 Anti- Rubella+
f 1 Cancer (undefined); 1 Basal Cell; 2 Bladder; 3 Breast; 3 Colon; 1 Gall Bladder, 1 Gastric/Adeno; 2 Liver; 1 Hepatoma; 3 lung; 1Pancreatic; 4 Rectal
g 19 Multitransfusion; 19 Multiparous; 15 Non-viral cirrhosis [Alcohol (6); Drug (3); Primary Biliary (6)]

As shown in Table 2, 99.83% of the random donor population (n=19,968) were initially nonreactive, 0.17% were initially reactive, and 0.13% were repeatedly reactive. Twenty-five (75.75%) of the 33 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 Peptide EIA was estimated from the results of screening tests in random U.S. and Canadian blood and plasma donors, and determined by the following formula:

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

Thus, assuming a zero prevalence rate of antibodies to HIV-1 and HIV-2 in this population, the Genetic Systems™ HIV-1/HIV-2 Peptide EIA has an estimated specificity of (19,968 - 25) x 100/ 19,968 = 99.87% (95% confidence interval[44]: 99.82 - 99.92%).

Six specimens from individuals with unrelated medical conditions were initially reactive. Four specimens (2 from individuals with elevated IgG, 1 from an individual with a positive ANA and 1 from an individual positive for antibodies to HTLV-I/II) were repeatedly reactive in the Genetic Systems™ HIV-1/HIV-2 Peptide EIA. Two specimens were negative for anti-HIV-1 by Western Blot and 2 were indeterminate. All 4 specimens were nonreactive for antibody to HIV-2 when tested with a licensed HIV-2 EIA. The 2 other initially reactive specimens (1 was anti-EBV positive and 1 was positive on a serological test for syphilis) were not repeatedly reactive. None of the remaining specimens from individuals with other medical conditions were reactive in the Genetic Systems™ HIV-1/HIV-2 Peptide EIA.

Sensitivity Studies

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

The reactivity of the Genetic Systems™ HIV-1/HIV-2 Peptide EIA was determined by testing serum and plasma samples from patients diagnosed as having AIDS (n = 309), and from 1850 individuals known to be HIV-1 antibody positive from U.S. (n=505) and non-U.S. locations (n= 1345)[a] for whom the clinical status was unknown. The samples utilized in the sensitivity evaluation of the assay were collected from diverse geographic regions, thereby increasing the likelihood of incorporating divergent strains of virus within the test population. Even though a diverse population has been tested with 100% sensitivity, it is not possible to ensure the detection of all possible divergent strains of HIV-1 or HIV-2. The results of testing are shown in Table 3.

Table 3: Reactivity in HIV-1 Known Positive Specimens

	Genetic Systems HIV-1/HIV-2 Peptide EIA		Licensed HIV-1/HIV-2 EIA	
Group	No. Repeatedly reactive	(% Reactive)	No. Repeatedly reactive	(% Reactive)
AIDS (n = 309)	309	(100%)	309	(100%)
Known Positive U.S. (n = 505)	505	(100%)	505	(100%)
Known Positive non-U.S. (n = 1345)[a]	1345	(100%)	1345	(100%)
Total	**2159**	**(100%)**	**2159**	**(100%)**

[a]Non-U.S. locations included the following: Canada (1,014); Central African Republic (100); Nigeria (56); Zimbabwe (53); Australia (49); Thailand (48); France (16); Ghana (5); Nairobi (4)

Of the 309 diagnosed AIDS patients, 100% were repeatedly reactive with the Genetic Systems™ HIV-1/HIV-2 Peptide EIA. Two hundred ten (210) of the AIDS specimens were positive on a licensed HIV-1 Western Blot. Western blot data is not available on the remaining 99 specimens, but they were repeatedly reactive on a second licensed HIV-1/HIV-2 EIA. All 99 specimens were considered to be positive for HIV given the diagnosis of AIDS for each patient. Of the known 1,850 positives from U.S. and non-U.S. locations, 1,342 were confirmed positive with one of three licensed HIV-1 Western blots; 508 were confirmed positive with an in-house HIV-1 Western blot.

The HIV-1 sensitivity of the Genetic Systems™ HIV-1/HIV-2 Peptide EIA was estimated from the results of testing 309 patients with AIDS. Studies demonstrated a positive test result in 309 of 309 patients for an estimated sensitivity of 100% (95% confidence interval[44]: 99.84 to 100%).

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

A total of 2,096 specimens from high risk individuals in the United States and Canada were tested with Genetic Systems™ HIV-1/HIV-2 Peptide EIA. Results of testing individuals from the United States (n = 1080) and Canada (n = 1,016) are shown in Table 4. The numbers include 800 specimens from U.S. STD clinic patients, 280 specimens prospectively collected at a U.S. hospital emergency room in a high HIV-1 prevalence area, and 1,016 specimens from Canadian homosexual males, injection drug users, and sex partners of known HIV positive persons. All specimens were screened with one or more FDA licensed and/or Canadian approved HIV-1/HIV-2 EIAs. All specimens repeatedly reactive with Genetic Systems™ HIV-1/HIV-2 Peptide EIA and/or the licensed/approved HIV-1/HIV-2 EIAs were tested with a licensed HIV-1 Western blot or in-house HIV-1 Western blot. Additionally, specimens tested in the United States that were repeatedly reactive with Genetic Systems™ HIV-1/HIV-2 Peptide EIA and/or the licensed HIV-1/HIV-2 EIA were tested with a licensed HIV-2 EIA. Specimens tested in Canada that were repeatedly reactive with Genetic Systems™ HIV-1/HIV-2 Peptide EIA and/or the licensed/approved HIV-1/HIV-2 EIA 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 investigational HIV-2 Western blot.

Table 4: Reactivity in Specimens from High-Risk Individuals from the United States and Canada

Group	No. Tested	Genetic Systems HIV-1/HIV-2 Peptide EIA Repeatedly Reactive	No. RR on one or more Licensed/ Approved HIV-1/HIV-2 EIAs	No. Pos. by HIV-1 Western Blot
U.S.	1,080	69 (6.4%)[a]	74[a]	59 (5.5%)
Canada	1,016	27 (2.7%)[b]	27[b]	26 (2.6%)
Total	**2,096**	**96 (4.6%)**	**101**	**85 (4.1%)**

[a]Sixty-two (62) specimens were repeatedly reactive on both the Genetic Systems™ HIV-1/HIV-2 Peptide EIA and one or more licensed HIV-1/HIV-2 EIAs.

[b]Twenty-six (26) specimens were repeatedly reactive on both the Genetic Systems™ HIV-1/HIV-2 Peptide EIA and one or more licensed/approved HIV-1/HIV-2 EIAs.

RR = Repeatedly Reactive

The Genetic Systems™ HIV-1/HIV-2 Peptide EIA detected 85/85(100%) of the HIV-1 confirmed positive specimens from high risk individuals in the United States and Canada.

A total of 83 specimens were additionally tested with a licensed HIV-2 EIA (62 specimens repeatedly reactive on Genetic Systems™ HIV-1/HIV-2 Peptide EIA and one or more licensed HIV-1/HIV-2 EIAs; 8 specimens repeatedly reactive on the Genetic Systems™ HIV-1/HIV-2 Peptide EIA only; 13 specimens repeatedly reactive on one or more licensed/approved EIAs only). Of the 83 specimens tested with a licensed HIV-2 EIA, 42 were repeatedly reactive. Of the 42 HIV-2 EIA repeatedly reactive specimens, 40 were confirmed positive for HIV-1. Two specimens required testing with an investigational HIV-2 Western blot. Both specimens were indeterminate on the HIV-2 Western blot.

Therefore, the Genetic Systems™ HIV-1/HIV-2 Peptide EIA detected all HIV confirmed positives in high risk populations from the United States and Canada.

Reactivity in Prospective Public Health Specimens

Results of testing prospective public health specimens with Genetic Systems™ HIV-1/HIV-2 Peptide EIA are summarized in Table 5. The data include 5,472[a] serum specimens tested at two Canadian locations. All specimens were screened with one or more FDA licensed and/or Canadian approved HIV-1/HIV-2 EIAs. Specimens repeatedly reactive with Genetic Systems™ HIV-1/HIV-2 Peptide EIA and/or the licensed/approved HIV-1/HIV-2 EIAs were tested with a licensed HIV-1 Western blot or in-house HIV-1 Western blot. Specimens that were repeatedly reactive with Genetic Systems™ HIV-1/HIV-2 Peptide EIA and/or the licensed/approved HIV-1/HIV-2 EIA 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 investigational HIV-2 Western blot.

Table 5: Detection of Antibodies to HIV-1 and/or HIV-2 in Prospective Public Health Specimens

	No. Tested	Genetic Systems HIV-1/HIV-2 Peptide EIA Repeatedly Reactive	No. RR on one or more Licensed/ Approved HIV-1/HIV-2 EIAs	No. Pos. by HIV-1 Western Blot
Site 1	3,057	27[b] (0.88%)	31[b] (1.01%)	22 (0.72%)
Site 2	2,415[a]	13[c] (0.54%)	24[c] (0.99%)	7 (0.29%)
Total	**5,472[a]**	**40 (0.73%)**	**55 (1.01%)**	**29 (0.53%)**

[a]The numbers include 45 specimens also tested in the high risk study.
[b]Twenty-three (23) specimens were repeatedly reactive on both the Genetic Systems™ HIV-1/HIV-2 Peptide EIA and one or more licensed/approved HIV-1/HIV-2 EIAs.
[c]Eight (8) specimens were repeatedly reactive on both the Genetic Systems™ HIV-1/HIV-2 Peptide EIA and one or more licensed HIV-1/HIV-2 EIAs.
RR = Repeatedly Reactive

The Genetic Systems™ HIV-1/HIV-2 Peptide EIA detected 29/29(100%) of the HIV-1 confirmed positive specimens from prospective public health populations.

A total of 35 specimens were additionally tested with a licensed HIV-2 EIA (2 specimens repeatedly reactive on Genetic Systems™ HIV-1/HIV-2 Peptide EIA and one or more licensed/approved HIV-1/HIV-2 EIAs; 9 specimens repeatedly reactive on the Genetic Systems™ HIV-1/HIV-2 Peptide EIA only; 24 specimens repeatedly reactive on one or more licensed/approved EIAs only). Of the 35 specimens tested with a licensed HIV-2 EIA, none were repeatedly reactive. No further HIV-2 confirmatory testing was performed.

Therefore, the Genetic Systems™ HIV-1/HIV-2 Peptide EIA detected all HIV confirmed positives in prospective public health populations from Canada.

Comparative sensitivity of the Genetic Systems™ HIV-1/HIV-2 Peptide EIA to a previously licensed test for antibody to HIV-1 and HIV-2 was evaluated in paired tests on high risk subjects [U.S. (n=1,080); Canadian (n=1,016)], prospective public health subjects (n=5,472) or known positive specimens from U.S. (n=505) and non-U.S. origin (n=1,345). In these studies, the Genetic Systems™ HIV-1/HIV-2 Peptide EIA was reactive for 1,964 of 1,964 subjects who had positive HIV-1/HIV-2 screening test results which had additionally been confirmed by HIV-1 Western blot.

Reactivity with HIV-1 Seroconversion Panels

The Genetic Systems™ HIV-1/HIV-2 Peptide EIA detected the presence of antibody to HIV-1 in specimens from 16 commercially available HIV-1 seroconversion panels as early as, or earlier than, a licensed HIV-1/HIV-2 EIA, and licensed HIV-1 Western blot. Reactivity demonstrated using one lot of the Genetic Systems™ HIV-1/HIV-2 Peptide EIA with 5 representative seroconversion panels is shown in Table 6 below. (Note: Only bleeds before and after the point of seroconversion are presented.)

Table 6: Detection of Antibody to HIV-1 in Representative Seroconversion Panels

Panel	Date of Bleed	Genetic Systems HIV-1/HIV-2 Peptide EIA	Licensed HIV-1/HIV-2 EIA	Licensed HIV-1 Western blot
PRB903	07/23/85	NR	NR	NEG
	07/25/85	NR	NR	IND
	07/30/85	R	NR	POS
	08/01/85	R	NR	POS
	08/06/85	R	R	POS
	08/08/85	R	R	POS
PRB914	01/12/90	R	NR	IND
	01/16/90	R	R	POS
	01/19/90	R	R	POS
PRB917	12/14/90	NR	NR	IND
	12/19/90	R	NR	IND
	12/21/90	R	NR	IND
	12/26/90	R	R	POS
PRB922	08/07/93	NR	NR	NEG
	08/11/93	NR	NR	NEG
	08/14/93	R	NR	NEG
	08/18/93	R	NR	POS
PRB924	12/13/93	NR	NR	NEG
	12/15/93	R	NR	NEG
	12/20/93	R	NR	POS

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

A total of 496 specimens, obtained from HIV-2 confirmed antibody positive individuals, were tested with Genetic Systems™ HIV-1/HIV-2 Peptide EIA. All specimens were found to be repeatedly reactive with a licensed HIV-2 EIA. Of the 496 specimens tested, all (100%) were classified as repeatedly reactive with Genetic Systems™ HIV-1/HIV-2 Peptide EIA; 261 of these specimens were confirmed as positive for antibody to HIV-1 and HIV-2 by Western blot; 235 specimens were positive on an investigational HIV-2 Western blot. (Of the 235 specimens, 231 were indeterminate and 4 were negative on a licensed HIV-1 Western blot.)

The HIV-2 sensitivity of the Genetic Systems™ HIV-1/HIV-2 Peptide EIA was determined by comparison with a previously licensed test for antibody to HIV-2. The Genetic Systems™ HIV-1/HIV-2 Peptide EIA and licensed HIV-2 EIA detected 496 of 496 samples which were additionally confirmed by a positive investigational HIV-2 Western blot, for an estimated sensitivity of 100% (95% confidence interval[44]: 99.9-100%) compared with Western blot.

Reactivity in Populations from an HIV-2 Endemic Area

The ability of the Genetic Systems™ HIV-1/HIV-2 Peptide EIA to detect antibodies to HIV-2 in specimens from an HIV-2 endemic area is shown in Table 7. The data include the following: 100 serum samples obtained from women attending a family planning clinic in Senegal; 617 serum samples collected from healthy adults and clinic patients in rural and urban areas of Liberia; 589 serum samples collected from low and high risk groups (including prostitutes) in Sierra Leone; and 287 serum samples collected prospectively in Côte d'Ivoire (risk group unknown). All samples were tested in parallel with a licensed HIV-1/HIV-2 EIA. The samples from Senegal, Sierra Leone and Liberia were also tested in parallel with a licensed HIV-2 EIA. Specimens from Côte d'Ivoire were tested with an investigational HIV-2 Western blot. Samples repeatedly reactive with the Genetic Systems™ HIV-1/HIV-2 Peptide EIA or the licensed HIV-1/HIV-2 EIA or HIV-2 EIA were tested with a licensed HIV-1 Western blot and an investigational HIV-2 Western blot. If the sample volume was not sufficient for testing with a licensed HIV-1 Western blot, it was tested with a licensed HIV-1 IFA.

Table 7: Detection of Antibodies to HIV-2 in Specimens from an Endemic Area

Endemic Area	Results with HIV-1/HIV-2 Peptide EIA: Number Tested	Results with HIV-1/HIV-2 Peptide EIA: Repeatedly Reactive	Licensed HIV-1/HIV-2 EIA Repeatedly Reactive	Licensed HIV-2 EIA Repeatedly Reactive	Repeatedly Reactive Specimens: Pos. by HIV-1 Western blot Alone	Pos. by HIV-2 Western blot Alone	Pos. by both HIV-1 HIV-2 Western blot
Senegal	100	3	1	2	1	0	0
Liberia	614[a]	46	*	*	2	3	0
Sierra Leone	589	66	75	84	28	5	4
Côte d'Ivoire	287	36	36	NT	17	3	7
Total	**1590**	**151**	**112**	**86**	**48**	**11**	**11**

*Samples were initially reactive on the licensed HIV-1/HIV-2 EIA (n=133) and the licensed HIV-2 EIA (n=124). There was insufficient volume for retesting.

[a]A total of 617 specimens were tested. However, 1 specimen was unresolved for HIV-1 and 2 specimens were unresolved for HIV-2. Therefore, these 3 specimens were not included in the total numbers. Two of the specimens were initially reactive with the licensed HIV-1/HIV-2 EIA and all three specimens were initially reactive with the licensed HIV-2 EIA.

Specimens were considered positive by HIV-2 Western blot if two of the following three bands were present: gp105/140, gp36/41, or p26. Specimens were considered positive by HIV-1 Western blot if two of the following three bands were present: gp120/160, gp41, or p24. A test specimen is interpreted as positive by licensed IFA when there is a specific cytoplasmic staining pattern in the HIV-1 infected cells and there is a significant difference in the intensity of fluorescent staining and the pattern of fluorescence between the HIV-1 infected and uninfected cells.

In this study, 9.5% (151/1590) of the specimens from West African populations that were tested by Genetic Systems™ HIV-1/HIV-2 Peptide EIA were repeatedly reactive. All of the specimens were also tested with a licensed HIV-1/HIV-2 EIA. One hundred twelve (112) specimens (7.0%) were repeatedly reactive. Specimens from Côte d'Ivoire were not tested with a licensed HIV-2 EIA but all were tested with an investigational HIV-2 Western blot. All specimens from Senegal, Liberia and Sierra Leone were tested with a licensed HIV-2 EIA (a total of 1303 specimens). Of the 1303 specimens tested, 86 specimens (6.6%) were repeatedly reactive on a licensed HIV-2 EIA.

All specimens testing repeatedly reactive by the Genetic Systems™ HIV-1/HIV2 Peptide EIA, licensed HIV-1/HIV-2 EIA and the licensed HIV-2 EIA were tested with a licensed HIV-1 Western blot or licensed HIV-1 IFA and an investigational HIV-2 Western blot, using the criteria given previously. All specimens positive by Western blot (48 specimens that were positive by HIV-1 Western blot, 11 specimens that were positve by HIV-2 Western blot, and 11 specimens that were positive by both HIV-1 and HIV-2 Western blot) and repeatedly reactive by a licensed HIV-1/HIV-2 EIA and/or licensed HIV-2 EIA were also repeatedly reactive by Genetic Systems™ HIV-1/HIV-2 Peptide EIA.

In addition, camparative sensitivity with a licensed HIV-1/HIV-2 EIA was evaluated in prospective studies in endemic areas of West Africa (Senegal, Liberia, Sierra Leone, Côte d'Ivoire). In these studies, the Genetic Systems™ HIV1/HIV-2 Peptide EIA was positive in 22 of 22 samples which were reactive by the licensed HIV-1/HIV-2 EIA and additionally confirmed by a positive investigational HIV-2 Western blot, demonstrating equivalent sensitivity for detection of antibody to HIV-2 compared with a previously licensed test.

PERFORMANCE CHARACTERISTICS OF CADAVERIC SPECIMEN TESTING

Reproducibility

Inter-assay reproducibility of Genetic Systems™ HIV-1/HIV-2 Peptide EIA was assessed using twenty post-mortem sera and twenty normal donor sera, spiked with HIV-1 and HIV-2 positive serum to give rectivity near the cutoff. Each of the samples was tested once on six different days on each of three lots of Genetic Systems™ HIV-1/HIV-2 Peptide EIA at one site. For inter-assay reproducibility over all lots, percent coefficient of variation (%CV) ranged from 5.1% to 11.8% for the spiked post-mortem samples and from 6.3% to 12.3% for the spiked normal donor samples.

Specificity

Specificity was evaluated in a clinical investigation at one site in two studies: 1. Fifty (50) paired pre- and post-mortem specimens were tested concurrently on

Genetic Systems™ HIV-1/HIV-2 Peptide EIA; 2. Sixty-five (65) post-mortem samples and sixty-five normal donor samples were tested concurrently on Genetic Systems™ HIV-1/HIV-2 Peptide EIA. Repeatedly reactive specimens were additionally tested with a licensed HIV-2 EIA and confirmed with a licensed HIV-1 Western blot and HIV-1 IFA. Results are presented in Tables 8 and 9 below.

Table 8:
Reactivity with Genetic Systems™ HIV-1/HIV-2 Peptide EIA

Population	Number Tested	Nonreactive	Initially Reactive	Repeatedly Reactive	Confirmed Positive
Pre-mortem	50	50 (100.00%)	0 (0.00%)	NA	NA
Post-mortem	50	50 (100.00%)	0 (0.00%)	NA	NA

NA = Not Applicable

Table 9:
Reactivity with Genetic Systems™ HIV-1/HIV-2 Peptide EIA

Population	Number Tested	Nonreactive	Initially Reactive	Repeatedly Reactive	Confirmed Positive
Post-mortem	65	65 (100.00%)	0 (0.00%)	NA	NA
Normal Donor	65	64 (98.46%)	1* (1.54%)	1* (1.54%)	UTI

NA = Not Applicable
UTI = Unable to interpret HIV-1 Western Blot
*This specimen was nonreactive on a licensed HIV-2 EIA and negative by HIV-1 IFA

Specificity of Genetic Systems™ HIV-1/HIV-2 Peptide EIA 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 one hundred fifteen (115) unselected post-mortem specimens were tested with the Genetic Systems™ HIV-1/HIV-2 Peptide EIA for determining specificity. Of the 115 specimens, 65 were tested and compared with 65 normal donor specimens while 50 post-mortem specimens were paired to pre-mortem specimens. None of the post-mortem specimens were reactive with the Genetic Systems™ HIV-1/HIV-2 Peptide EIA. Thus, the Genetic Systems™ HIV-1/HIV-2 Peptide EIA has an estimated specificity of 100.00% (95%; binomial confidence interval[1] = [99.57%, 100%]) for post-mortem specimens. By comparison, a total of sixty-five normal donor specimens were tested concurrently with the sixty-five post-mortem specimens. One normal donor specimen (1.54%) was initially and repeatedly reactive. The specimen was unable to be interpreted on the licensed

HIV-1 Western blot due to high background. This specimen was also tested with the licensed Fluorognost HIV-1 IFA test kit from Waldheim Pharmazeutika and found to be IFA negative. Additionally, the specimen was tested with the Genetic Systems™ HIV-2 EIA and found to be nonreactive. The mean optical density signal for the 65 post-mortem specimens was 0.043 whereas the mean optical density signal for the 65 normal donor specimens was 0.054. In this population, according to the Student's t-test, there is no significant statistical difference between the post-mortem mean optical density signal and that of the normal donors (assuming unequal variance). Additionally, 50 pre-mortem specimens were tested concurrently with 50 paired post-mortem specimens. The mean optical density signal for the post-mortem specimens was 0.066 whereas the mean optical density signal for the pre-mortem specimens was 0.063. According to the Student's t-test, there is no significant statistical difference between the post-mortem mean optical density signal and that of the pre-mortem (paired two-specimen for means).

Sensitivity

Ninety-one (91) post-mortem samples and ninety-one (91) 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-one spiked and unspiked post-mortem samples were tested concurrently with 91 spiked and unspiked normal donor specimens on the same run of Genetic Systems™ HIV-1/HIV-2 Peptide EIA. Spiked specimens were expected to be reactive and therefore were not retested in duplicate. Results are presented in Table 10 below.

Table 10
Reactivity with Genetic Systems™ HIV-1/HIV-2 Peptide EIA

Population	Number Tested	Nonreactive	Initially Reactive	Repeatedly Reactive	Confirmed Positive
Spiked post-mortem	91	0 (0.00%)	91 (100.00%)	NT	91 (100.00%)
Unspiked post-mortem	91	91 (100.00%)	0 (0.00%)	NA	NA
Spiked normal donor	91	0 (0.00%)	91 (100.00%)	NT	91 (100.00%)
Unspiked normal donor	91	91 (100.00%)	0 (0.00%)	NA	NA

NT = Not Tested; NA = Not Applicable

As can be seen in Table 10, of ninety-one post-mortem samples and ninety-one normal donor samples, spiked at a potency near cutoff and tested concurrently, all (100.00%) were reactive with Genetic Systems™ HIV-1/HIV-2 Peptide EIA.

Furthermore, according to the Student's t-test, there is no significant statistical difference between the spiked post-mortem mean optical density signal and that of the spiked normal donor mean optical density signal (two sample assuming unequal variances). 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.

BIBLIOGRAPHY

1. DesJarlis DC, Marmor M, Cohen H, et al: Antibodies to a retrovirus etiologically associated with acquired immunodeficiency syndrome (AIDS) in populations with increased incidence of the syndrome. **Morbidity Mortality Weekly Rep** 33:377-379, 1984.

2. Delmonico FL, Snydman DR: Organ donor screening for infectious diseases. **Transplantation** 65 (5): 603-610. 1998.

3. 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.

4. 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.

5. Coffin J, Haase A, Levy JA, et al: What to call the AIDS virus? **Nature** 321:10, 1986.

6. Gallo RC, Montagnier L: AIDS in 1988. **Scientific American** 259: 40-48, 1988.

7. Greene WC: The molecular biology of Human Immunodeficiency Virus Type 1 infection. **New Engl J Med** 324: 308-317, 1991.

8. Clavel F, Guetard D, Brun-Vezinet F: Isolation of a new human retrovirus from West African patients with AIDS. **Science** 233:343-346, 1986.

9. Clavel F, Manshino K, Chameret S, et al: Human immunodeficiency virus type 2 infection associated with AIDS in West Africa. **New Engl J Med** 316: 1180-1185, 1987.

10. De Cock KM, Brun-Vezinet F: Epidemiology of HIV-2 infection. **AIDS 1989** 3:S89-S95, 1989.

11. Centers for Disease Control: Testing for Antibodies to Human Immonodeficiency Virus Type 2 in the U.S. **MMWR** 41:1-9, 1992.

12. Hoff R, Weiblen BJ, Schwerzler M, et al: Specific antibodies to HIV-2 detected in an anonymous newborn blood specimen from Massachusetts. **Fourth Consensus Conference on Testing for Human Retroviruses,** March 1989.

13. Ayanian JZ, Maguire JH, Marlink RG, et al: HIV-2 infection in the United States. **New Engl J Med** 320:1422-1423, 1989.

14. O'Brien TR, George JR, Holmberg SD: Human immunodeficiency virus type 2 infection in the United States. **JAMA** 267 : 2775-2779, 1992.

15. Centers for Disease Control: AIDS due to HIV-2 infection - New Jersey. **Morbidity and Mortality Weekly Rep** 37: 33-35, 1988.

16. Reported at the Tenth Annual Conference on Human Retrovirus Testing, Reno NV, March 6-9, 1995.

17. Brun-Vezinet F, Katlama C, Roulot D, et al: Lymphadenopathy associated virus type 2 in AIDS and AIDS-related complex. **Lancet** 1:128-132, 1987.

18. Quinn TC, Zacarias FRK, St. John RK: AIDS in the Americas: an emerging public health crisis. **New Engl J Med** 320:1005-1007, 1989.

19. Guyader M, Emerman M, Sonigo P, et al: Genome organization and transactivation of the human immunodeficiency virus type 2. **Nature** 326:662-669, 1987.

20. Cabrian K, Shriver K, Goldstein L, et al: Human immunodeficiency virus type 2: a review. **J Clinical Immunoassay** 11:107-114, 1988.

21. George JR, 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.

22. Starcich BR, Hahn BH, Shaw GM, et al: Identification and characterization of conserved and variable regions in the envelope gene of HTLV-III/LAV, the retrovirus of AIDS. **Cell** 45: 637-648, 1986.

23. Wang JJG, Steel S, Wisniewolski R, Wang CY: Detection of antibodies to human T-lymphotrophic virus type III by using a synthetic peptide of 21 amino acid residues corresponding to a highly antigenic segment of gp41 envelope protein. **Proc Nat Acad Sci** USA 83: 6159-6163, 1986.

24. Cosand WL: Synthetic antigen for the detection of AIDS-related disease. U.S. Patent #4,629,783, 1986.

25. Fenouillet E, Sorensen A-M, Lacroix M, Coutellier A, Herson S, Fretz-Foucault C, Gluckman J-C: Early and specific diagnosis of seropositivity to HIVs by an enzyme-linked immunosorbent assay using *env*-derived synthetic peptides. **AIDS** 4: 1137-41, 1990.

26. Gnann JW, McCormick, Mitchell S, Nelson J, Oldstone MBA: Synthetic peptide immunoassay distinguishes HIV type 1 and HIV type 2 infections. **Science** 237: 1346-1349, 1987.

27. Gnann JW, Nelson JA, Oldstone MBA: Fine mapping of an immunodominant domain in the transmembrane glycoprotein of human immunodeficiency virus. **J Virol** 61: 2639-2641, 1987.

28. Alizon M, Wain-Hobson S, Montagnier L, Sonigo P: Genetic variability of the AIDS virus: nucleotide sequence analysis of two isolates from African patients. **Cell** 46: 63-74, 1986.

29. Bos ES, van der Doelen AA, van Rooy N, et al: 3, 3', 5, 5'-tetramethylbenzidine as an ames test negative chromogen for horseradish peroxidase in enzyme immunoassay. **J Immunoassay** 2:187-204, 1981.

30. Garner RC, Walpole AL, Rose FL: Testing of some benzidine analogues for microsomal activation to bacterial mutagens. **Cancer Letters** 1:39-42, 1975.

31. 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.

32. 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.

33. 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.

34. Sehulster 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.

35. NCCLS: Preparation and testing of reagent water in the clinical laboratory - second edition; approved guideline. **National Committee for Clinical Laboratory Standards, document C3-A2**, 11(13), (ISBN1-562328-127-X), 1991.

36. Centers for Disease Control: Update on acquired immune deficiency syndrome (AIDS)-United States. **MMWR** 31:507-508, 1982.

37. Centers for Disease Control, Revision of the case definition of acquired immunodeficiency syndrome for national reporting-United States. **MMWR** 34:373-375, 1985.

38. Taylor JMG, Schwartz K, Detels R: The time from infection with human immunodeficiency virus (HIV) to the onset of AIDS. **J Infect Dis** 154:694-697, 1986.

39. Hunter DJ, De Gruttola V: Estimation of risk of outcomes of HTLV-III infection. **Lancet** 1:677-678, 1986.

40. Rutherford GW, Lifson AR, Hessol NA, et al: Course of HIV Infection in a cohort of homosexual and bisexual men: an 11 year follow up study. **Br Med J** 301:1183-1188, 1990.

41. Carlson JR, Bryant ML, Hinrichs SH, et al: AIDS serology testing in low and high-risk groups. **JAMA** 253:3405-3408, 1985.

42. Schumacher RT, Garret PE, Tegtmeier GE, Thomas D: Comparative detection of anti-HIV in early HIV seroconversion. **J Clin Immunoassay** 11: 130-134, 1988.

43. Wara, DW, Luzuriaga K, Martin NL, et al: Maternal transmission and diagnosis of human immunodificiency virus during infancy. **Annals NY Acad Sci** 693: 14-19, 1993.

44. Blythe CR, Still HA: Binomial confidence intervals, **Journal of the American Statistical Association**, 78:108-116, 1983.

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