Summary Basis of Approval

Reference Nos.:	96-0959 (PLA Supplement) 96-0830 (ELA Supplement)
Applicant:	Genetic Systems Corporation 6565 185th Avenue NE Redmond, WA 98052
Proper Name:	Human Immunodeficiency Virus Type 1
Product Trade Name:	Genetic Systems™ rLAV EIA

I. INDICATIONS FOR USE

The Genetic Systems rLAV EIA is an *in vitro* qualitative enzyme immunoassay for the detection of circulating antibodies to Human Immunodeficiency Virus Type 1 in human serum, plasma, or dried blood spots. The rLAV EIA is intended to be used as a screening test for donated blood or plasma and as an aid in the diagnosis of infection with HIV-1.

II. BRIEF DESCRIPTION OF TEST

The Genetic Systems rLAV EIA is manufactured using both a strain of HIV-1 designated LAV, which is propagated in a CEM cell line, and an *E. coli* recombinant containing antigenic regions of the HIV-1 envelope protein gp41. Microwell plates are coated with a mixture of HIV-1 viral lysate and recombinant protein.

During the assay, specimens are evaluated for the presence of HIV-1 antibodies by interaction with the adsorbed antigens in the wells. Samples to be tested are diluted in Specimen Diluent and added to each well, and the plate is incubated and washed. If antibodies to HIV-1 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 absorbances of controls and specimens are determined with a spectrophotometer with wavelength set at 450 nm.

Components of the rLAV EIA are listed below:

- HIV-1 Coated Microwell Plates: Plates containing 96 wells coated with adsorbed HIV-1 antigens. Preservative: Proclin 300™.
- Negative Control: Human serum or plasma non-reactive for HBsAg and antibodies to HIV-1, HIV-2, HTLV-I, and HCV. Preservatives: 0.005% Gentamicin sulfate and 0.5% Proclin 300.
- HIV-1 Positive Control: Human serum or plasma containing HIV-1 immunoglobulin, specific for HIV-1 by EIA; Non-reactive for HBsAg and for antibodies to HCV and HTLV-I. Preservatives: 0.005% Gentamicin sulfate and 0.5% Proclin 300.
- Specimen Diluent: Buffered solution with normal bovine serum. Preservative: 0.1% Proclin 300.
- Conjugate Concentrate: Solution of goat anti-human IgM and IgG, horseradish peroxidase-conjugated. Preservative: 0.0001% Thimerosal.
- Conjugate Diluent: Buffered solution with protein stabilizers (normal goat serum and normal bovine serum). Preservative: 0.1% Proclin 150.
- Wash Solution Concentrate (30X): Contains sodium chloride and Tween 20.
- Chromogen Reagent: Contains tetramethylbenzidine (TMB) and dimethylsulfoxide (DMSO).
- Chromogen Diluent: Contains hydrogen peroxide, citric acid, and dimethylsulfoxide (DMSO).
- Stopping Reagent: 1N H_2SO_4.
- Plate Sealers: Used to cover the plates during testing.

III. MANUFACTURING AND CONTROLS

A. Manufacturing

The rLAV EIA is manufactured using both a strain of HIV-1 designated LAV, which is propagated in a CEM cell line, and an *E. coli* recombinant containing antigenic regions of the HIV-1 envelope protein gp41. Both the viral lysate and recombinant protein used in the assay are evaluated for purity and functionality. Each lot of coated plates, which contains the viral lysate and recombinant peptide, is tested against a panel of monoclonal antibodies and human reference sera to ensure appropriate reactivity in the rLAV EIA.

Positive and Negative Controls are prepared from human sera which are positive and negative, respectively, for antibody to HIV. The material used to make the HIV-1 Positive Control is specific for HIV-1 by EIA. Both the positive and negative sera are non-reactive for HBsAg, anti-HTLV-I, and anti-HCV. Positive serum is heat-treated to eliminate the infectivity of any HIV that might be present.

Raw materials intended for use in the product are subject to appropriate quality control evaluations before they are accepted for use in manufacturing. The quality of the product component is assessed at multiple stages during manufacture, using tests for product appearance and performance. Components are assembled into test kits, each lot of which is subjected to a final performance test that includes evaluation with Reference Panels prepared by the Center for Biologics Evaluation and Research (CBER) and by Genetic Systems Corporation. The panels contain serum specimens from donors negative for antibody to HIV-1, and from individuals positive for HIV-1 antibody. The performance test measures potency, reproducibility, sensitivity, and specificity. All lots of components of the Genetic Systems rLAV EIA (except for Stopping Reagent) are also monitored for bioburden (microbial load) and must meet specifications.

B. Stability Studies

The stability of the Genetic Systems rLAV EIA reagents at the recommended storage condition of 2-8°C have been verified by periodic evaluation of three (3) lots maintained under these conditions for a minimum of twelve (12) months. These studies indicate that no compromise in efficacy of kit performance is apparent under these conditions. Environmental stress studies have also been performed to determine the stability of the kit when frozen, thawed, and exposed to elevated temperature and humidity. Data from these studies support a 12-month dating period for the rLAV EIA, based on the stability of the component with the shortest dating period.

C. Methods of Validation

Product purity and potency is assured through Quality Control assessment of product appearance and performance. Product performance is assessed through laboratory evaluations comparing each lot to a control lot using panels produced by CBER and Genetic Systems Corporation. The panels contain specimens from some individuals who are negative for antibody to both HIV-1 and HIV-2, and from individuals positive for HIV-1 antibody. All components of the Genetic Systems rLAV EIA, except the Stopping Reagent, are tested for bioburden levels and must meet pre-established criteria. All validation tests are performed by Genetic Systems Corporation. Three master lots of the Genetic Systems rLAV EIA have been submitted to CBER for evaluation. Each master lot of the product, along with protocols summarizing pertinent product testing, will be submitted for evaluation and approval by CBER prior to release for distribution.

D. Labeling

The labeling, including container and package labels and the package circular, have been reviewed for compliance with 21 CFR 610.60, 610.61, 610.62, and 809.10 and were found to be satisfactory. The package circular for the rLAV EIA states that the intended use of the test is for detection of HIV-1 antibodies in serum, plasma, or dried blood spots. The product trade name, Genetic Systems rLAV EIA, is not known to conflict with other biologic or device trade names.

E. Establishment Inspection

An inspection of the manufacturing facilities used to produce the Genetic Systems rLAV EIA was conducted June 9, 1997 through June 13, 1997. Facilities and procedures are currently in compliance with cGMPs.

F. Environmental Assessment (EA)

A detailed Environmental Impact Analysis Report was provided in the Product License Application (Ref. No. 96-0959) for the Genetic Systems rLAV EIA. Procedures taken by Genetic Systems Corporation to assure that no adverse environmental impacts occur are listed below.

1. The human serum containing antibody to HIV-1, which is used as a positive control in the rLAV EIA kit, is heat-inactivated before further manufacturing. In addition, human serum for the positive and negative controls must be shown to be free of hepatitis B surface antigen and antibody to HCV and HTLV-I before it can be used in the manufacture of this product.

2. Appropriate precautionary statements for users are included in the package insert for the product. These statements include instructions for the safe handling and disposal of hazardous materials.

3. Product shipping containers are appropriately labeled and are shipped according to applicable regulations.

Genetic Systems Corporation is in compliance with applicable emissions requirements (including occupational), at the federal, state, and local levels. There are no adverse environmental impacts anticipated as a result of this product licensure.

IV. BIOLOGICAL PRINCIPLES OF THE TEST

Genetic Systems rLAV EIA is for the detection of circulating antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) in human serum, plasma, or dried blood spots, and is indicated as a screening test and as an aid in the diagnosis of infection with HIV-1. The assay is manufactured using both a strain of HIV-1 designated LAV, which is propagated in a CEM cell line, and an *E. coli* recombinant containing antigenic regions of the HIV-1 envelope protein gp41. The CEM 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 prior to coating the microwell plates. The recombinant gp41 is expressed in *E. coli* and purified from cell lysate prior to coating the microwell plates. Microwell plates are coated with a mixture of the HIV-1 viral lysate and the recombinant protein.

Samples to be tested are diluted in Specimen Diluent and added to each well, and the plate is incubated and washed. If antibodies to HIV-1 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.

The detection of antibodies is visualized by the addition of a Working Chromogen Solution, which is next 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. The absorbance of each specimen is compared to the absorbance of the known positive and negative controls, and calculations of the ratio of the specimen absorbance / cutoff value determine if the sample is negative or reactive for antibodies to HIV-1.

V. CLINICAL DATA

A. Performance Characteristics of Serum and Plasma Testing

1. Reproducibility

Inter-assay and intra-assay reproducibility were determined by testing a panel of 8 specimens. The panel consisted of one (1) HIV-1 positive plasma specimen, 5 serial dilutions of this HIV-1 positive specimen (diluted past the cutoff in negative plasma) and 2 negative plasma specimens. Each of the 8 panel members was tested in triplicate on 5 runs of each of 3 lots. The data were analyzed at Genetic Systems according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS)[1,2]. The mean optical density (OD), standard deviation (SD), and percent coefficient of variation (%CV) for each panel member are listed in Table 1 below.

Table 1: Reproducibility of the Genetic Systems rLAV EIA

Inter-assay Reproducibility					Intra-assay Reproducibility				
Specimen	N*	Mean OD	SD[1]	%CV	Specimen	N*	Mean OD	SD[2]	%CV
1	366	2.037	0.188	9.23%	1	366	2.037	0.079	3.89%
2	366	1.642	0.161	9.82%	2	366	1.642	0.073	4.42%
3	366	0.657	0.084	12.79%	3	366	0.657	0.040	6.05%
4	366	0.306	0.043	14.06%	4	366	0.306	0.024	7.81%
5	366	0.158	0.023	14.73%	5	366	0.158	0.013	8.49%
6	366	0.088	0.013	14.87%	6	366	0.088	0.009	9.78%
7	365*	0.030	0.006	18.78%	7	365*	0.030	0.003	9.63%
8	366	0.030	0.005	17.74%	8	366	0.030	0.003	10.42%

* Outliers not included in statistical calculations

1. NCCLS Vol. 12 No. 4, p.33 Eq's 12 and 13
2. NCCLS Vol. 12 No. 4, p.32 Eq 11

2. Specificity Studies

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

The results of testing specimens from random blood donors and specimens from individuals with medical conditions unrelated to HIV-1 are summarized in Table 2. The data include 4,756 serum and 3,537 plasma samples obtained from 8,293 donors at 5 geographically distinct locations and 340 specimens from individuals with various medical conditions.

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

Group	Results obtained with Genetic Systems rLAV EIA			
	Number tested	Non Reactive	Initially Reactive	Repeatedly Reactive
Random Donors Site 1[a]	1759 (100.00%)	1756 (99.83%)	3 (0.17%)	1 (0.06%)
Random Donors Site 2[a]	1504 (100.00%)	1502 (99.87%)	2 (0.13%)	2 (0.13%)
Random Donors Site 3[a]	1500 (100.00%)	1500 (100.00%)	0 (0.00%)	0 (0.00%)
Random Donors Site 4[a]	2000 (100.00%)	2000 (100.00%)	0 (0.00%)	0 (0.00%)
Random Donors Site 5[a]	1530 (100.00%)	1528 (99.87%)	2 (0.13%)	0 (0.00%)
TOTAL	**8293 (100.00%)**	**8286 (99.92%)**	**7 (0.08%)**	**3 (0.04%)**
Bacterial/Parasitic Diseases[b]	40 (100.00%)	40 (100.00%)	0 (0.00%)	0 (0.00%)
Autoimmune Diseases[c]	80 (100.00%)	80 (100.00%)	0 (0.00%)	0 (0.00%)
Other Viral Diseases[d]	140 (100.00%)	140 (100.00%)	0 (0.00%)	0 (0.00%)
Malignancies[e]	20 (100.00%)	20 (100.00%)	0 (0.00%)	0 (0.00%)
Other Specimens[f]	60 (100.00%)	60 (100.00%)	0 (0.00%)	0 (0.00%)
TOTAL	**340 (100.00%)**	**340 (100.00%)**	**0 (0.00%)**	**0 (0.00%)**

a. Serum was tested at sites 1(1752 sera), 2 and 3; plasma was tested at sites 1 (7 plasma), 4 and 5.
b. 20 Toxoplasmosis; 20 Syphilis
c. 20 Rheumatoid factor +; 20 SLE / ANA +; 20 Elevated IgG; 20 Elevated IgM
d. 20 HBsAg +; 9 Anti-HTLV-I +; 4 Anti-HTLV-I/II +; 7 Anti-HTLV-II +; 20 Anti-CMV +; 20 Anti-EBV +; 20 Anti-HCV +; 20 Anti-HSV +; 20 Anti-Rubella +
e. 1 Cancer (undefined); 1 Basal Cell; 2 Bladder; 2 Breast; 3 Colon; 1 Gall Bladder; 1 Gastric/Adeno; 2 Liver; 3 Lung; 1 Lymph; 2 Rectal; 1 Leukemia / RA
f. 20 Multi-Transfusion; 20 Multiparous; 20 Cirrhosis [Alcohol (6); Drug (5); Unknown (9)]

In a random population, 99.92% were initially nonreactive, 0.08% were initially reactive, and 0.04% were repeatedly reactive, as shown in Table 2. Three (3) of 7 (42.86%) of the initially reactive specimens were repeatedly reactive upon retesting. None of the repeatedly reactive specimens was positive for antibodies to HIV-1 by a licensed HIV-1 Western blot. None of the specimens from individuals with other medical conditions was reactive in the Genetic Systems rLAV EIA .

Specificity of the Genetic Systems rLAV EIA was estimated from the results of screening tests in random U.S. blood and plasma donors. Specificity was estimated by the following formula:

$$\frac{(\text{\# normal donor specimens} - \text{\# repeatedly reactive specimens}) \times 100}{(\text{\# normal donor specimens} - \text{\# repeatedly reactive specimens confirmed positive for antibodies to HIV-1})}$$

A total of 8,293 donor specimens were tested; 3 of these specimens were repeatedly reactive by the rLAV EIA. Of the repeatedly reactive specimens, none was confirmed to be positive for antibodies to HIV-1. Thus the Genetic Systems rLAV EIA has an estimated specificity of 99.96% (95% confidence interval: 99.91-100.00%) in serum and plasma.

3. Sensitivity Studies

Reactivity of Known HIV-1 Positive Specimens

The sensitivity of the Genetic Systems rLAV EIA was determined by testing serum and plasma samples from patients diagnosed as having AIDS (n = 201), ARC (n = 20), HIV-1 Asymptomatic (n = 26) and from 498 individuals known to be HIV-1 antibody positive from U.S. (n = 300) and non-U.S. locations (n = 198[a]) for whom the clinical status was unknown. The results of testing are shown in Table 3.

Table 3: Reactivity of HIV-1 Known Positive Specimens

Group	Results Obtained with Genetic Systems rLAV EIA			
	Number Tested	Nonreactive	Initially Reactive	Repeatedly Reactive
AIDS	201 (100%)	0 (0.00%)	201 (100%)	201 (100%)
ARC	20 (100%)	0 (0.00%)	20 (100%)	20 (100%)
HIV-1 Asymptomatic	26 (100%)	0 (0.00%)	26 (100%)	26 (100%)
Known HIV-1 Positive - U.S	300 (100%)	0 (0.00%)	300 (100%)	300 (100%)
Known HIV-1 Positive - Non - U.S.[a]	198 (100%)	0 (0.00%)	198 (100%)	198 (100%)
Total	745 (100%)	0 (0.00%)	745 (100%)	745 (100%)

[a] Non U.S. locations included the following: Central African Republic (n = 42); Nigeria (n = 40); Zimbabwe (n = 9); Australia (n = 41); Thailand (n = 41); Ghana (n = 4); Nairobi (n = 1); Sierra Leone (n = 20).

All (100%, 745/745) known HIV-1 positive specimens were repeatedly reactive on the Genetic Systems rLAV EIA. All of the known positives were confirmed positive with a licensed HIV-1 Western blot.

Sensitivity of the Genetic Systems rLAV EIA was estimated from the results of testing 201 patients with AIDS. Studies demonstrated a positive test result in 201 of 201 patients for an estimated sensitivity of 100% (95% confidence interval: 99.75 to 100%). Additionally, sensitivity to antibody to HIV-1 was evaluated in subjects with ARC (n = 20), HIV-1 positive asymptomatic (n = 26), and known positive specimens from the U.S. (n = 300) and outside the U.S. (n = 198). In these studies, the rLAV EIA was positive with 544 of 544 subjects with a positive HIV-1 screening test which were additionally confirmed with a licensed HIV-1 Western blot.

Reactivity of Specimens from High-Risk Individuals

The results of testing for antibodies to HIV-1 in 500 sera / plasma specimens prospectively collected from individuals at high risk for HIV-1 infection are shown in Table 4. The numbers include 300 plasma specimens from prisoners, 100 sera from STD clinic patients, and 100 sera collected from patients at a hospital emergency room in a high HIV-1 prevalence area. All specimens were also screened with a licensed HIV-1 EIA. All specimens which were repeatedly reactive with the Genetic Systems rLAV EIA and/or the licensed HIV-1 EIA were tested with a licensed HIV-1 Western blot.

Table 4: Reactivity of Prospective Specimens from High-Risk Individuals

Group	Results Obtained with Genetic Systems rLAV EIA				Repeatedly Reactive Specimens
	Number Tested	Nonreactive	Initially Reactive	Repeatedly Reactive	Positive by licensed HIV-1 Western blot
Prisoners	300 (100.00%)	288 (96.00%)	12 (4.00%)	12 (4.00%)	11
STD Clinic	100 (100.00%)	86 (86.00%)	14 (14.00%)	14 (14.00%)	14
E.R. Patients	100 (100.00%)	83 (83.00%)	17 (17.00%)	17 (17.00%)	16
Total	**500 (100.00%)**	**397 (79.40%)**	**43 (8.60%)**	**43 (8.60%)**	41

The Genetic Systems rLAV EIA detected 11/11 (100%) of the HIV-1 confirmed positive specimens from prisoners, 14/14 (100%) of the HIV-1 confirmed positives from STD patients, and 16/16 (100%) of the HIV-1 confirmed positives from hospital emergency room patients in a high prevalence area.

Reactivity with HIV-1 Seroconversion Panels

Sensitivity of the Genetic Systems rLAV EIA was also compared with previously licensed tests for antibody to HIV-1 using commercially available HIV-1 seroconversion panels and was equivalent or better, based on time of appearance of antibodies.

The Genetic Systems rLAV EIA detected the presence of antibody to HIV-1 in specimens from 30 commercially available HIV-1 seroconversion panels as early as, or earlier than, a licensed HIV-1 EIA and HIV-1 Western blot. Of the 30 panels tested, Genetic Systems rLAV EIA detected 19 of the panels at an earlier bleed and 11 of the panels at the same bleed when compared to the licensed HIV-1 EIA. Twelve examples of the reactivity demonstrated by the rLAV EIA on seroconversion samples are shown in Table 5 below.

Table 5: Detection of Antibody to HIV-1 in Representative Seroconversion Panels

Panel member	First Bleed Detected as Repeatedly Reactive / Positive					
	Genetic Systems rLAV EIA	Licensed Recombinant-based HIV-1 EIA	Licensed Viral Lysate-based HIV-1 EIAs			Licensed HIV-1 Western Blot*
			HIV-1 EIA # 1*	HIV-1 EIA # 2*	HIV-1 EIA # 3	
PRB909	2	3	3	3	3	3
PRB914	1	1	1	4	4	4
PRB917	5	5	6	7	7	7
PRB919	2	2	2	UD**	UD**	2
PRB924	6	6	7	8	UD**	8
PRB925	5	5	5	6	6	UD**
PRB927	3	3	3	4	5	4
PRB930	4	4	4	UD**	UD**	UD**
PRB931	6	6	7	7	8	7
CPI-2	6	NA**	6	7	7	7
SVO-0231	C	C	E	E	F	D
SVO-0241	D	D	D	E	F	D

*Data from certificates of analysis from panel vendors.
**UD: Undetected as positive/reactive at any bleed
***NA: Not available

B. Performance Characteristics of Dried Blood Spots

1. Specificity Studies

Reactivity of Dried Blood Spot Specimens from Random Blood Donors and Neonates

The results of testing dried blood spot specimens collected on filter paper from random blood donors and neonates with Genetic Systems rLAV EIA are summarized in Table 6 below. The data include 2,525 dried blood spot specimens (DBS) obtained from individuals at three geographically distinct locations.

Table 6: Detection of Antibodies to HIV-1 in Dried Blood Spot Specimens in Neonates and Random Blood Donors

Group	Results obtained with Genetic Systems rLAV EIA			
	Number tested	Non Reactive	Initially Reactive	Repeatedly Reactive
Adults Site 1	744 (100.00%)	744 (100.00%)	0 (0.00%)	0 (0.00%)
Adults Site 2	780 (100.00%)	779 (99.87%)	1 (0.13%)	0 (0.00%)
TOTAL ADULTS	**1524 (100.00%)**	**1523 (99.93%)**	**1 (0.07%)**	**0 (0.00%)**
Neonates Site 5	**1001 (100.00%)**	**996 (99.50%)**	**5 (0.50%)**	**4 (0.40%)**

NA = Not Applicable

As shown in Table 6, 99.93% of the DBS from adult random donors were initially nonreactive, 0.07% were initially reactive and none (0.00%) were repeatedly reactive. Of the 1,001 neonate DBS, 99.50% were initially nonreactive, 0.50% were initially reactive, and 0.40% were repeatedly reactive upon retesting. Four of 5 initially reactive specimens were

repeatedly reactive upon retesting. None of the repeatedly reactive specimens was positive for antibodies to HIV-1 on an investigational HIV-1 Western blot.

Specificity of the Genetic Systems rLAV EIA when testing dried blood spot specimens (DBS) was estimated from neonates and adults, using the following formula:

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

A total of 1,524 dried blood spot specimens collected from random blood donors was tested: none of these specimens was repeatedly reactive by the rLAV EIA. Thus the Genetic Systems rLAV EIA has an estimated specificity of 100.00% (95% confidence interval: 99.97 to 100.00%) in testing adult DBS specimens.

A total of 1,001 DBS specimens collected from neonates was tested: four of these specimens were repeatedly reactive by the rLAV EIA. Of the repeatedly reactive specimens, none was confirmed to be positive for antibodies to HIV-1. Thus the Genetic Systems rLAV EIA has an estimated specificity of 99.60% (95% confidence interval: 99.16 to 100.00%) in testing neonatal DBS specimens.

2. Sensitivity Studies

Reactivity of Known HIV-1 Positive DBS Specimens

The reactivity of known HIV-1 positive dried blood spots (DBS) on Genetic Systems rLAV EIA was determined by testing paired or simulated DBS from patients diagnosed as having AIDS (n = 101), ARC (n = 20), HIV-1 positive asymptomatic (n = 26) and from individuals known to be HIV-1 antibody positive from U.S. (n = 50) and non-U.S. locations (n = 50[a]). Results of testing are shown in Table 7 below.

Table 7: Reactivity of Known HIV-1 Positive DBS Specimens

Group	Results with Genetic Systems rLAV EIA		
	Number Tested	Sample	Repeatedly Reactive
AIDS	101 (100%)	DBS (paired) Serum/Plasma	101 (100%) 101 (100%)
ARC	20 (100%)	DBS (paired) Serum/Plasma	20 (100%) 20 (100%)
HIV-1 Asymptomatic	26 (100%)	DBS (paired) Serum/Plasma	26 (100%) 26 (100%)
Known HIV-1 Positive - U.S	50 (100%)	DBS (simulated) Serum/Plasma	50 (100%) 50 (100%)
Known HIV-1 Positive - Non - U.S.[a]	50 (100%)	DBS (simulated) Serum/Plasma	50 (100%) 50 (100%)
Total	247 (100%)	DBS Serum/Plasma	247 (100%) 247 (100%)

[a] Non U.S. locations included the following: Central African Republic (8); Nigeria (7); Zimbabwe (7); Australia (8); Thailand (8); Ghana (4); Nairobi (1); Sierra Leone (7)

All (100%, 247/247) of the known HIV-1 positive DBS and serum or plasma samples were repeatedly reactive when tested on the Genetic Systems rLAV EIA. All of the serum or plasma samples were confirmed positive with a licensed HIV-1 Western blot.

Sensitivity of the Genetic Systems rLAV EIA was estimated from results of testing dried blood spot specimens collected from subjects with AIDS (n = 101), ARC (n = 20), HIV-1 positive asymptomatic (n = 26), and known HIV-1 positive specimens from subjects in the U.S. (n = 50) and outside the U.S. (n = 50). In these studies rLAV EIA was positive with 247 of 247 subjects (100%) with a positive HIV-1 EIA screening test. In addition, the paired serum/plasma specimens were confirmed with a licensed Western blot. All of these specimens (n = 247) were paired with serum/plasma and demonstrated 100% correlation for detection of HIV-1 antibody.

Reactivity of DBS Specimens from High-Risk Individuals

The results of testing 333 dried blood spots (DBS) for antibodies to HIV-1 with Genetic Systems rLAV EIA in specimens prospectively collected from individuals at high risk for HIV-1 infection are shown in Table 8. The numbers include 25 simulated DBS specimens (paired with serum or plasma) from patients at a hospital emergency room in a high HIV-1 prevalence area, 75 simulated DBS (paired with serum or plasma) from STD clinic patients, and 233 unpaired DBS collected from prostitutes (n = 53), IV drug users (n = 56), individuals in an alcohol treatment center (n = 45), and homosexual / bisexual males (n = 79). All DBS and simulated DBS specimens were also tested with a licensed HIV-1 EIA. Simulated dried blood spot specimens repeatedly reactive with the Genetic Systems rLAV EIA or the licensed HIV-1 EIA were compared to the sera / plasma result on a licensed HIV-1 Western blot. Unpaired dried blood spot specimens repeatedly reactive with Genetic Systems rLAV EIA or the licensed EIA were tested on an investigational HIV-1 Western blot. All specimens that confirmed positive and were detected as repeatedly reactive by the licensed HIV-1 EIA were also detected as repeatedly reactive by the Genetic Systems rLAV EIA.

Table 8: Reactivity of Dried Blood Spot Specimens from High-Risk Individuals

Group	Results with Genetic Systems rLAV EIA			Repeatedly Reactive Specimens
	Number Tested	Sample	Repeatedly Reactive	Positive by licensed HIV-1 Western blot [a]
E.R. patients from high prevalence area	25 (100%)	DBS (simulated) Serum/Plasma	2 (8.00%) 2 (8.00%)	2
STD Clinic Patients	75 (100%)	DBS (simulated) Serum/Plasma	9 (12.00%) 9 (12.00%)	9
Total	100 (100%)	DBS (simulated) Serum/Plasma	11 (11.00%) 11 (11.00%)	11
Prostitutes	53 (100%)	DBS (unpaired)	2 (3.77%)	2
IV Drug Users	56 (100%)	DBS (unpaired)	2 (3.57%)	1
Alcohol Treatment Patients	45 (100%)	DBS (unpaired)	2 (4.44%)	0
Homosexual / Bisexual Males	79 (100%)	DBS (unpaired)	4 (5.06%)	3[b]
Total	233 (100%)	DBS (unpaired)	10 (4.29%)	6

NT = Not tested

[a] Serum/plasma samples tested with a licensed HIV-1 Western blot. DBS tested with an investigational HIV-1 Western blot.

[b] Three of 4 unpaired DBS, repeatedly reactive on Genetic Systems rLAV EIA, were positive when tested on an investigational HIV-1 Western blot. One specimen QNS for testing on an investigational HIV-1 Western blot.

Sensitivity of the Genetic Systems rLAV EIA was also estimated from results of dried blood spot specimens from high risk individuals including E.R. patients from a high prevalence area (n = 25), STD clinic patients (n = 75), prostitutes (n = 53), IV drug users (n = 56), alcohol treatment patients (n = 45), and homosexual/bisexual males (n = 79). In these studies the rLAV EIA was repeatedly reactive with 17 of 17 subjects with a repeatedly reactive HIV-1 EIA screening test which were additionally confirmed by immunoblot. Eleven of these specimens were paired with serum/plasma and demonstrated 100% correlation for detection of HIV-1 antibody.

Thus, the Genetic Systems rLAV EIA, when testing dried blood spot specimens, has an estimated sensitivity of 100% (95% confidence interval: 99.81% to 100%) in high-risk settings.

Reactivity of Simulated DBS Specimens from Seroconversion Panels

Testing of simulated dried blood spots prepared from 30 commercially available seroconversion panels resulted in sensitivity performance substantially equivalent to that observed with serum/plasma (Table 5). Of the 30 panels tested, Genetic Systems rLAV EIA detected 18 of the panels at an earlier bleed and 12 of the panels at the same bleed when compared to the licensed HIV-1 EIA.

SBA Genetic Systems rLAV EIA (96-0959)
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for Mary Gustafson
Director
Division of Blood Applications

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

June 29, 1998

Our Reference Number: 96-0959

Mr. Reed W. Simmons
Genetic Systems Corporation
6565 185th Avenue NE
Redmond, WA 98052-5039

Dear Mr. Simmons:

The Center for Biologics Evaluation and Research (CBER) has completed its review of your request to supplement your product license application for Human Immunodeficiency Virus Type 1, to include the manufacture and sale in interstate and foreign commerce a new HIV-1 enzyme immunoassay (EIA) which contains a combination of HIV-1 viral lysate and HIV-1 recombinant antigen, i.e., the Genetic Systems rLAV EIA. This in vitro qualitative enzyme immunoassay is to be used for the detection of antibodies to Human Immunodeficiency Virus Type 1 in human serum, plasma, and dried blood spots.

Based upon the review of information provided in support of your request, the supplement has been found acceptable.

You are requested to submit samples of each future master lot of the product test kit together with protocols consisting of a summary of essential manufacturing data inclusive of all applicable test results. No master lots of the product test kit shall be distributed until notification of release is received from the Director of CBER.

The expiration dating for the Genetic Systems rLAV EIA is 12 months when stored at 2-8° C. Any request to extend this dating period must be accompanied by the results of ongoing stability studies.

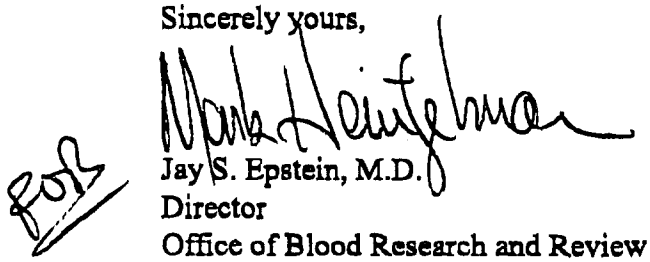
Any lot of Genetic Systems rLAV EIA found to fall outside of the approved specifications, including expiration dating periods, should be withdrawn from the market. In addition, any reports of significant product defects or product complaints concerning the use of the Genetic Systems rLAV EIA should be submitted to the Office of Compliance, CBER, HFM-650.

A copy of your labeling submission for the Genetic Systems rLAV EIA is enclosed. Please submit three copies of final printed labeling at the time of use and include part II of the label transmittal form (Form FDA 2567) with completed implementation information.

In addition, please submit three copies of the proposed introductory advertising and promotional labeling. You may wish to submit the proposed materials in draft form with an FDA form 2567 to CBER, Advertising and Promotional Labeling Staff, HFM-202. Promotional claims should be consistent with and not contrary to approved labeling. No comparative claims or claim of superiority over other similar products should be made unless data to support such claims are submitted to and approved by CBER.

This information will be placed on file with your product license application for Human Immunodeficiency Virus Type 1.

Sincerely yours,



Jay S. Epstein, M.D.
Director
Office of Blood Research and Review
Center for Biologics Evaluation
and Research

Enclosures

HUMAN IMMUNODEFICIENCY VIRUS TYPE I (Viral Lysate and *E. coli* Recombinant Antigen)

Genetic Systems™ rLAV EIA

Enzyme Immunoassay (EIA) for the Detection of Antibody to Human Immunodeficiency Virus Type 1 (HIV-1) in Human Serum, Plasma, or Dried Blood Spots. The rLAV EIA is intended to be used as a screening test for donated blood or plasma and as an aid in the diagnosis of infection with HIV-1.

For in vitro diagnostic use

32511 • 480 Tests
32510 • 960 Tests
32513 • 4800 Tests

Manufactured By:
Genetic Systems Corporation
U.S. License No. 978

Revised: March 1998

A subsidiary of:

NAME AND INTENDED USE

Genetic Systems™ rLAV EIA is the Genetic Systems Corporation Enzyme Immunoassay for detection of circulating antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) in human serum, plasma, or dried blood spots. The rLAV EIA is intended to be used as a screening test for donated blood or plasma and as an aid in the diagnosis of infection with HIV-1.

SUMMARY AND EXPLANATION OF THE TEST

The acquired immunodeficiency syndrome (AIDS) is caused by viruses transmitted by sexual contact, exposure to blood (including sharing contaminated needles and syringes) or certain blood products, or transmitted from an infected mother to her fetus or child during the perinatal period.[1] Human Immunodeficiency Virus Type 1 (HIV-1) has been isolated from patients with AIDS and AIDS-related complex (ARC), and from healthy persons at high risk for AIDS.[2,3] The prevalence of antibodies specific for HIV-1 in AIDS and ARC patients and persons at increased risk for AIDS is high. The incidence of infection with HIV-1 in people not known to be at increased risk is not known. The Genetic Systems™ rLAV enzyme-linked immunoassay (EIA) was developed to detect antibodies to HIV-1 and to identify potentially infectious units of donated blood and plasma. It has been established that repeatedly reactive units of blood and plasma should be eliminated from the blood supply.[4,5]

In order to afford maximum protection of the blood supply, the Genetic Systems™ rLAV EIA was designed to be extremely sensitive. As a result, non-specific reactions may be seen in samples from some people who, due to prior pregnancy, blood transfusion, or other exposure, have antibodies to the human cells or media in which the HIV-1 virus used for manufacture of the Genetic Systems™ rLAV EIA is grown.[6] Because of these and other nonspecific reactions, it is appropriate to investigate specimens found to be reactive on Genetic Systems™ rLAV EIA with an assay that gives improved predictability that antibody to HIV-1, in fact, is present. When a specimen reads in an initial test (is initially reactive), the Genetic Systems™ rLAV EIA should be repeated in duplicate on the same specimen. Reactivity in either or both of these duplicate tests (repeatedly reactive) is highly predictive of the presence of antibody in people at increased risk for infection (e.g., homosexual men, hemophiliacs, or intravenous drug users). Repeatedly reactive specimens obtained from people at increased risk for infection with HIV-1 are usually found to contain antibodies when evaluated by additional more specific, or supplemental tests. However, when the EIA is used to screen populations in which the prevalence of infection with HIV-1 is low (e.g., blood donors), nonspecific reactions may be more common. Information about prevalence of infections with HIV-1 in persons in various categories of risk, as well as clinical and public health guidelines, is available in the publication Morbidity and Mortality Weekly Reports.

For clinical and public health applications of the Genetic Systems™ rLAV EIA, both the degree of risk for infection with HIV-1 of the person studied and the degree of reactivity of their serum may be of value in interpreting the test; however, these correlations are imperfect. Therefore, in most settings it is appropriate to investigate reactive specimens by additional more specific, or supplemental, tests such as Western Blot.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The Genetic Systems™ rLAV EIA is manufactured from both a strain of HIV-1 designated LAV, which is propagated in a CEM cell line, and an *E. coli* recombinant containing antigenic regions of the HIV-1 envelope protein gp41. 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 prior to coating the microwell plates. The recombinant gp41 is expressed in *E. coli* and purified from cell lysate prior to coating the microwell plates. The purified, inactivated virus and recombinant gp41 are adsorbed onto wells of a microwell plate.

During the assay, specimens are evaluated for the presence of HIV-1 antibodies by interaction with the adsorbed virus and recombinant protein in the wells. Samples to be tested are diluted in Specimen Diluent and added to each well, incubated with the adsorbed antigen, and washed. If antibodies to the virus are present, they bind to the 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 set at 450 nm wavelength.

REAGENTS

Genetic Systems™ rLAV EIA Product Description
Product No: 32511 (480 Tests), 32510 (960 Tests), 32513 (4800 Tests)

Component	Contents	Preparation
R1 • HIV-1 Coated Microwell Plates, 5, 10, or 50	•Microwell strips coated with HIV-1 antigens (viral lysate and *E. coli* recombinant) •Proclin 300™	Use as supplied.
C0 •rLAV Negative Control, 1 or 5 vial(s) (1.8 ml)	•Normal human serum •Nonreactive for HBsAg •Nonreactive for antibody to HIV-1, HIV-2, HTLV-I/II and HCV. •0.005% Gentamicin sulfate •0.5% Proclin 300™	Dilute in Specimen Diluent as described.
C1 •rLAV Positive Control, 1 or 5 vial(s) (1.6 ml)	•Human serum containing HIV-1 immunoglobulin •Nonreactive for HBsAg •Nonreactive for antibody to HCV and HTLV-I/II •0.005% Gentamicin sulfate •0.5% Proclin 300™	Dilute in Specimen Diluent as described.
R2 • rLAV EIA Specimen Diluent 3, 5, or 25 bottle(s) (120 ml)	•Normal bovine serum •0.1% Proclin 300™	Ready to use as supplied.
R3 • rLAV EIA Conjugate Concentrate, 1 or 5 vial(s) (1.8 ml)	•Solution of goat anti-human IgM and IgG horseradish peroxidase conjugate •0.5% Proclin 300™	Dilute in Conjugate Diluent as described.
R4 • rLAV EIA Conjugate Diluent 1 or 5 bottle(s) (120 ml)	•Normal goat serum •Normal bovine serum •0.1% Proclin 150™	Ready to use as supplied.
R5 • Wash Solution Concentrate(30X), 2 or 10 bottle(s) (120 ml)	•Sodium chloride •Tween 20	Dilute to working concentration with distilled or deionized water. Clinical laboratory reagentwater Type I or Type II is acceptable.
R6 • Chromogen Reagent, 1 or 5 vial(s) (1.5 ml)	•Tetramethylbenzidine (TMB)* •Dimethylsulfoxide (DMSO)	Dilute with EIA Chromogen Diluent as described.
R7 • Chromogen Diluent, 1 or 5 bottle(s) (120 ml)	•Hydrogen peroxide •Citric acid •Dimethylsulfoxide (DMSO)	Ready to use as supplied.
R8 • Stopping Reagent, 1 or 5 bottle(s) (120 ml)	• 1N H_2SO_4	Ready to use as supplied.
• Plate Sealers	•25 sealers each	Ready to use as supplied.

*Note: Tetramethylbenzidine is a non-carcinogenic and non-mutagenic chromogen for peroxidase.[7,8]

Store the kit at 2-8°C. Bring all reagents except rLAV 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.

WARNINGS FOR USERS
For In Vitro Diagnostic Use

WARNING: FDA has licensed this test for use with serum, plasma and dried blood spot 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 Positive Control is heat-treated and the virus is inactivated using a chaotropic agent and heat. However, handle all the reagents as though capable of transmitting infection. All tests should be conducted in the same manner and 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 that do not contain 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.[9-11] 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.[11]

PRECAUTIONS FOR USERS

1. Do not use the kit beyond the stated expiration date.

2. The only reagents that may be used with different lots of the rLAV EIA or other Genetic Systems test kits are the Chromogen Reagent, Chromogen Diluent, Wash Solution Concentrate and Stopping Reagent. Do not mix any other reagents from different lots.

3. Do not use the Chromogen Diluent for the 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, polypropylene container for the Working Conjugate Solution. Do not use a polystyrene container. Exposure of the Conjugate Diluent or Conjugate Concentrate to sodium azide or serum will inactivate Conjugate Solution.

6. Avoid exposing the 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. Avoid contact of Stopping Reagent with any oxidizing agent. Do not allow Stopping Reagent to come into contact with metals.

8. Use clean, polypropylene containers to prepare and store the Working Chromogen Solution. Do not use polystyrene containers. If glassware must be used, pre-rinse thoroughly with 1 N sulfuric or hydrochloric acid followed by at least three washes of deionized water. Be sure that no acid residue remains on the glassware.

9. Bring all reagents except 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 the Negative and Positive Controls in the same manner as patient specimens.

12. If a specimen or reagent is inadvertently not added to a well, the assay result will read negative.

13. Inadequate adherence to package insert instructions may result in erroneous results.

14. Use only adequately calibrated equipment with this assay.

REAGENT PREPARATION AND STORAGE

Working Specimen Diluent

Bring Specimen Diluent to room temperature. Invert Specimen Diluent to thoroughly mix before 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 adding 10µl of Conjugate Concentrate to 1 ml of Conjugate Diluent (refer to following table). Note Concentrate lot number, date and time of preparation, and time of expiration on the container. Use a clean, polypropylene container for the Conjugate Solution. Do not use a polystyrene container. 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.

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 10µl of Chromogen Reagent to 1 ml of Chromogen Diluent (refer to following table). Note Chromogen Reagent lot number, date and time of preparation, and time of expiration on the container. 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 very pale 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 and prepare fresh reagent in a clean, polypropylene container. Do not use a polystyrene container.

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

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 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 the 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 by adding one part Wash Solution Concentrate (30X) to 29 parts of deionized or distilled water (e.g., 120ml of Wash Solution Concentrate to 3480 ml of 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 up to four weeks in a plastic container. Note the lot number, date prepared, and expiration date on the container. Discard if no suds are evident in the Working Wash Solution. Prepare a sufficient quantity of Working Wash Solution to complete a full run.

SPECIMEN COLLECTION, PREPARATION AND STORAGE

Serum or Plasma

Serum or plasma may be used. The following anticoagulants have all been evaluated and found to be acceptable: EDTA, heparin, sodium citrate, CPDA-1, and ACD. Samples which are collected into anticoagulant tubes should be filled as labeling indicates to avoid improper dilution. Remove the serum or plasma from the clot or red cells to avoid hemolysis. Specimens with observable particulate matter should be clarified by centrifugation prior to testing. No clinically significant effect on assay results has been detected in samples with increased levels of protein, lipids, bilirubin, or hemoglobin, or after heat inactivation of patient samples.

Serum or plasma may be stored at 2-8°C for up to seven 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 temperatures 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). Refrigerate samples at 2-8°C at receipt, or freeze for longer storage.

This kit is not licensed for use with specimens other than serum, plasma, and dried blood spots. This kit is not intended for use on saliva/oral fluids or urine samples.

Collection of Dried Blood Spots

In addition to detecting antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) in human serum or plasma, the Genetic Systems™ rLAV EIA may be used to test whole blood specimens collected onto filter paper and dried. Drops of whole blood should be obtained according to the National Committee for Clinical Laboratory Standards for the collection of diagnostic specimens using skin puncture, by either finger puncture or heel stick.[12]

1. Label a separate piece of filter paper for each specimen with the appropriate specimen identification. Use a ball point pen or other water-indelible marker. Handle the filter paper by the edges; do not touch the areas that will be used to collect specimens.

2. Prepare the area (either finger or heel) for puncture. The puncture must be performed with sufficient force and penetration to sustain a flow of at least several drops of blood. Allow a large drop of free-flowing blood to collect at the puncture site. Touch the filter paper to the edge of the drop to collect the drop, and allow another large drop to form at the puncture site. Continue to collect drops in this manner until the wound ceases to bleed or until collection is sufficient.

3. Collect each drop of blood in a separate area of the filter paper. If the paper is marked with several circles, place each drop in a different circle. *Do not layer successive drops of blood in the same spot.* **Note: At least two spots of blood, each ≥ 1/4 inch in diameter, must be provided so that sufficient sample is available for the initial EIA test, a repeat EIA test in duplicate (if necessary), and an additional more specific or supplemental test (if necessary). The Genetic Systems™ rLAV EIA should not be performed on a DBS specimen unless sufficient sample (at least two spots of blood, each ≥ 1/4 inch in diameter) is provided to run these tests.**

4. If the wound stops flowing before sufficient blood has been obtained, a second puncture should be performed. The wound may be massaged *very gently* to encourage formation of large blood droplets. Do not squeeze the wound to obtain more blood as this may result in hemolysis of the specimen or mixture of other body fluids with the specimen.

5. After the blood has been absorbed into the filter paper, it should be dried at room temperature for at least three hours. The filter paper may be allowed to dry at room temperature overnight. When dry, the spots will be a uniform dark brown. No areas of red coloration should be seen; the appearance of the spots should be similar to that of a dried blood stain.

When the blood spots are completely dry, a sample may be punched and eluted as described below. If dried blood specimens are to be shipped, they should be enclosed and sealed in either a moisture barrier container, such as a heavy-duty, zip-lock bag with desiccant,[12] or a high quality bond envelope.[13]

Dried Blood Spot Specimen Preparation

1. Use a 1/4" paper punch to **remove a 1/4" disk of each whole blood specimen** to be tested. Punch the disk from a uniform area of one of the completely dried spots of blood. **Place each 1/4" disk in a separate well of a clean, uncoated microwell plate.**

2. Use a precision pipette or repeating dispenser to **add 200 µl of Specimen Diluent to each well containing a filter paper disk. Mix the specimens well** to wet the filter paper thoroughly (for example, tap plate several times or use a plate shaker or rotator). Assure that all filter paper disks are immersed in the Specimen Diluent. Cover the microwell plate with a plate sealer to minimize evaporation. **Elute the specimens for 2 hours at room temperature on a shaking platform or overnight at 2-8°C.**

3. At the end of the two hour elution or when the refrigerated eluates **warm to room temperature, mix** until the filter paper disk is almost white (a faint brown color may remain in the disk). Specimens eluted in this manner must be diluted by an additional factor of 1:2.5 prior to testing with the Genetic Systems™ rLAV EIA (see step 6 of rLAV EIA Procedure).

Storage of Dried Blood Spots

Studies performed at Genetic Systems indicate that completely dried specimens may be stored frozen (-20°C) or refrigerated (2-8°C) for at least two months under low humidity conditions. Storage under elevated temperature conditions (room temperature or 37°C) leads to increasing non-specific reactivity over time. Therefore, it is recommended that dried blood spots be stored for no more than one week at room temperature, or up to two months refrigerated or frozen; routine storage at elevated temperatures is not recommended. If specimens are stored at any conditions other than the ones listed above, the user must validate the stability of the specimens under those storage conditions. If specimens are to be stored in a humid environment (≥ 60% relative humidity), the user should include a desiccant.

rLAV EIA PROCEDURE FOR SERUM AND PLASMA OR DRIED BLOOD SPOT ELUATES

Materials Required

See Reagents Section on page 4.

Materials Required but not Provided

1. Precision pipettes (to deliver 5 µl, 10µl, 100 µl, 1 ml, 5 ml, and 10 ml) or automated pipettor-dilutor, and pipette tips.

2. Appropriate containers to prepare diluted specimens and reagents.

3. Dry-heat incubator capable of maintaining 37 ± 1°C.

4. Genetic Systems microwell plate or strip washer or an equivalent. The washer must be capable of dispensing at least 300µl per well and cycling 5 times.

5. 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 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.

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.

9. Use clean, polypropylene containers for preparation of Working Conjugate and Chromogen Solutions. Do not use polystyrene containers.

10. Deionized or distilled water. Clinical laboratory reagent water Type I or Type II is acceptable.

11. Gloves.

12. Laboratory timer.

13. EIA reagent reservoirs (optional).

Additional Materials Required For Dried Blood Spot Testing

1. Cotton fiber filter paper (ie. Schleicher and Schuell #903 specimen collection paper)
2. Lancets
3. 1/4" paper punch
4. Clean, uncoated microwell plates
5. Optional shaker or rotator capable of at least 120 rpm
6. Zip-lock plastic bags or high quality bond envelope
7. Desiccant

Preliminary Statements

1. The expected run time for this procedure is approximately 3-4 hours from initiation of the first incubation step. 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 patient samples is determined by the controls on each individual plate.

3. The number of controls to be included on each plate in each run of this assay is two 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

Serum or plasma specimens are diluted 1:101 in Specimen Diluent prior to testing. Dried blood spot eluates are diluted 1:2.5 (see Step 6 below) in Specimen Diluent prior to testing.

1. **Bring all of the reagents except the Conjugate Concentrate to room temperature** before beginning the assay procedure.

2. Prepare Working Wash Solution, Working Conjugate Solution, and Working Chromogen Solution. See Reagent Preparation Section.

3. Remove any strips from the microwell plates not needed for the assay and replace with labeled null strips.

4. 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.

5. **For Serum or Plasma and Controls:** Dilute specimens and controls 1:101 in the Specimen Diluent. (For example, dilute 5 µl of specimen in 500 µl of Specimen Diluent.) Two separate dilutions of Positive Control and three separate dilutions of Negative Control should be assayed with each plate or partial plate of specimens. Mix all diluted specimens and controls thoroughly. Mix gently to avoid foaming of the diluent. All microwell plates containing controls and specimens must be subjected to the same process and incubation times. Add 200 µl of the diluted serum or plasma specimen or control to the appropriate well.

6. **For Dried Blood Spots:** Add 60 µl of Specimen Diluent and 40 µl of dried blood spot eluate directly to the appropriate well, and mix.

7. 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 ± 1°C.**

8. At the end of the incubation period, carefully remove the plate cover and aspirate the fluid from each well into a biohazard container. **Wash the microwell plate or strip a minimum of five times** with the Wash Solution (at least 300 µl/well/wash). Aspirate the Wash Solution after each wash. After the last wash, aspirate the liquid completely or blot the inverted plate on absorbent paper towels. Note: Grasp the plate holder firmly at the center of the long sides before inverting to blot.

9. **Add 100 µl of Working Conjugate Solution to each well containing a specimen or control.**

10. 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 ± 1°C.**

11. 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 300 µl/well/wash). Aspirate the Wash Solution after each wash. After the last wash, aspirate the liquid completely or blot the inverted plate on absorbent paper towels. Note: Grasp the plate holder firmly at the center of the long sides before inverting to blot.

12. **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 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.)

13. 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.

14. **Read absorbance within 30 minutes** after adding the Stopping Reagent, using the 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

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.[11]

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 0.000 AU and less than or equal to 0.100 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.

Example:

Negative Control Sample Number	Absorbance
1	0.034
2	0.025
3	0.046
	0.105

$$\frac{\text{Total absorbance}}{3} = \frac{0.105}{3} = 0.035 \text{ (NCx)}$$

Mean Positive Control Absorbance Value (PCx)

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

Example:

Positive Control Sample Number	Absorbance
1	1.435
2	1.501
	2.936

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

The PCx must be greater than or equal to 1.000 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 Positive Control absorbance values above are within the range of 0.65 to 1.35 times the PCx as shown by the following calculation:

$$0.65 \times (PCx) = 0.65 \times 1.468 = 0.954$$
$$1.35 \times (PCx) = 1.35 \times 1.468 = 1.982$$

Therefore, the acceptable range is 0.954 to 1.982.

Cutoff Value

The cutoff value is the NCx plus 0.250.

Example:

$$NCx = 0.035$$
$$\text{Cutoff Value} = 0.035 + 0.250 = 0.285$$

Validity Criteria

A plate run is valid if the following criteria are met:

1. The individual Negative Control AU values are greater than 0.000 AU and less than or equal to 0.100 AU. (One Negative Control value may be discarded if it is outside this range. The mean of the Negative Controls (NCx) may be calculated from the two remaining values.)

2. The mean absorbance of the Positive Control is equal to or greater than 1.000 AU, and each Positive Control value is within the reproducibility range of 0.65 to 1.35 times the mean of the Positive Controls (neither Positive Control 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) is invalid. The samples on that plate(s) should be run again.

INTERPRETATION OF RESULTS

The presence or absence of antibodies to HIV-1 is determined by relating the absorbance value of the specimen to the cutoff value. The cutoff value is determined by adding 0.250 to the mean absorbance value of the Negative Controls.

1. Specimens with absorbance values less than the cutoff value are considered non-reactive by the Genetic Systems™ rLAV EIA and may be considered negative for antibody to HIV-1. 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™ rLAV EIA and should be re-diluted and retested in duplicate before interpretation. If, after repeat testing, the absorbance of either or both duplicate specimens is greater than or equal to the calculated cutoff, the specimen is considered repeatedly reactive. Those samples 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.

5. If the specimen is repeatedly reactive, the probability that antibodies to HIV-1 are present is high, especially in specimens obtained from subjects at increased risk for HIV-1 infection or in 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 HIV-1 Western blots or HIV-1 immunofluorescence assays (IFA).

   Specimens that are repeatedly reactive by the rLAV EIA and are found to be positive for HIV-1 by additional more specific or supplemental testing are considered to be positive for antibodies to HIV-1.

   The interpretation of results of specimens found repeatedly reactive by rLAV EIA and negative or indeterminate on additional, more specific testing for antibodies to HIV-1 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™ rLAV EIA Procedure and the Interpretation of Results must be followed closely when testing for the presence of antibodies to HIV-1 in plasma or serum, or whole blood on filter paper. Data regarding the interpretation were derived from testing serum or plasma samples or whole blood on filter paper collected from individual subjects. 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™ rLAV EIA detects circulating antibodies to HIV-1 and thus is useful in screening blood and plasma donated for transfusion and further manufacture, in evaluating patients with signs or symptoms of AIDS, and in establishing prior infection with HIV-1. Clinical studies continue to clarify and refine the interpretation and medical significance of the presence of antibodies to HIV-1.[14,15] 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 should be considered an important part of testing for antibody to HIV-1 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.[16,17] Testing alone cannot be used to diagnose AIDS, even if the recommended investigation of reactive specimens suggests a high probability that antibodies to HIV-1 are 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.

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.[18,19] However, in a prospective study, AIDS developed in 51% of homosexual men after 10 years of infection.[20]

8. Data obtained from testing persons both at increased and at low risk for HIV-1 infection suggest that repeatedly reactive specimens with high reactivity on the rLAV EIA may be more likely to demonstrate the presence of antibodies to HIV-1 by additional more specific or supplemental testing.[21] Borderline reactivity is more frequently nonspecific, especially in samples obtained from persons at low risk for infection with HIV-1;

however, the presence of antibodies to HIV-1 in some of these specimens can be demonstrated by additional more specific or supplemental testing.

9. It is generally recognized that detection of HIV antibody in infants born to seropositive mothers is not adequate to diagnose HIV infection in the infant, since maternal IgG frequently persists for as long as 18 months after birth. Supplemental assays designed specifically for neonatal specimens may be helpful in resolving such cases.[22]

10. 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.

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. 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 testing a panel of 8 specimens. The panel of 8 members consisted of one (1) HIV-1 positive plasma specimen, 5 serial dilutions of this HIV-1 positive plasma specimen (diluted past the cutoff in negative plasma), and 2 negative plasma specimens. Each of the 8 panel members was tested in triplicate on 5 runs of each of 3 lots. The data were analyzed at Genetic Systems according to the National Committee for Clinical Laboratory Standards (NCCLS)[a,b]. The mean optical density (OD), standard deviation (SD), and percent coefficient of variation (%CV) for each panel member are listed in Table 1 below.

Table 1: Reproducibility of the Genetic Systems ™ rLAV EIA

Inter-assay Reproducibility					Intra-assay Reproducibility				
Specimen	N	Mean OD	SD[a]	%CV	Specimen	N	Mean OD	SD[b]	%CV
1	366	2.037	0.188	9.23%	1	366	2.037	0.079	3.89%
2	366	1.642	0.161	9.82%	2	366	1.642	0.073	4.42%
3	366	0.657	0.084	12.79%	3	366	0.657	0.040	6.05%
4	366	0.306	0.043	14.06%	4	366	0.306	0.024	7.81%
5	366	0.158	0.023	14.73%	5	366	0.158	0.013	8.49%
6	366	0.088	0.013	14.87%	6	366	0.088	0.009	9.78%
7	365*	0.030	0.006	18.78%	7	365*	0.030	0.003	9.63%
8	366	0.030	0.005	17.74%	8	366	0.030	0.003	10.42%

*Outlier 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

SPECIFICITY AND SENSITIVITY
Specificity Studies

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

The results of testing specimens from random blood donors and specimens from individuals with medical conditions unrelated to HIV-1 infection are summarized in Table 2. The data include 4,756 serum and 3,537 plasma samples obtained from 8,293 donors at five geographically distinct locations, and 340 specimens from individuals with various medical conditions.

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

Group	Results Obtained with Genetic Systems™ rLAV EIA			
	Number Tested	Non-Reactive	Initially Reactive	Repeatedly Reactive
Random Donors, Site 1[a]	1759 (100.00%)	1756 (99.83%)	3 (0.17%)	1 (0.06%)
Random Donors, Site 2[a]	1504 100.00%)	1502 (99.87%)	2 (0.13%)	2 (0.13%)
Random Donors, Site 3[a]	1500 (100.00%)	1500 (100.00%)	0 (0.00%)	0 (0.00%)
Random Donors, Site 4[a]	2000 (100.00%)	2000 (100.00%)	0 (0.00%)	0 (0.00%)
Random Donors, Site 5[a]	1530 (100.00%)	1528 (99.87%)	2 (0.13%)	0 (0.00%)
TOTAL:	**8293 (100.00%)**	**8286 (99.92%)**	**7 (0.08%)**	**3 (0.04%)**
Bacterial/Parasitic Diseases[b]	40 (100.00%)	40 (100.00%)	0 (0.00%)	0 (0.00%)
Autoimmune Diseases[c]	80 (100.00%)	80 (100.00%)	0 (0.00%)	0 (0.00%)
Other Viral Diseases[d]	140 (100.00%)	140 (100.00%)	0 (0.00%)	0 (0.00%)
Malignancies[e]	20 (100.00%)	20 (100.00%)	0 (0.00%)	0 (0.00%)
Other Specimens[f]	60 (100.00%)	60 (100.00%)	0 (0.00%)	0 (0.00%)
TOTAL:	**340 (100.00%)**	**340 (100.00%)**	**0 (0.00%)**	**0 (0.00%)**

a Serum was tested at sites 1 (1752 sera), 2 and 3; plasma was tested at sites 1 (7 plasma), 4 and 5.

b 20 Toxoplasmosis, 20 Syphilis

c 20 Rheumatoid factor positive; 20 SLE/ANA positive; 20 Elevated IgG; 20 Elevated IgM.

d 20 HBsAg+; 9 Anti-HTLV-I+; 7 Anti-HTLV-II+; 4 Anti-HTLV-I/II+; 20 Anti-CMV+; 20 Anti-EBV+; 20 Anti-HCV+; 20 Anti-HSV+; 20 Anti-Rubella+.

e 1 Cancer (undefined); 1 Basal Cell; 2 Bladder; 2 Breast; 3 Colon; 1 Gall Bladder; 1 Gastric/Adeno; 2 Liver; 3 Lung; 1 Lymph; 2 Rectal; 1 Leukemia/RA

f 20 Multi-transfusion; 20 Multiparous; 20 Cirrhosis [Alcohol (6); Drug (5); Unknown (9)]

In a random donor population 99.92% were initially nonreactive, 0.08% were initially reactive and 0.04% were repeatedly reactive, as shown in Table 2. Three of 7 (42.86%) of the initially reactive specimens were repeatedly reactive upon retesting. None of the repeatedly reactive specimens was positive for antibodies to HIV-1 by a licensed HIV-1 Western blot. None of the specimens from individuals with other medical conditions was reactive in the Genetic Systems™ rLAV EIA.

Specificity of the Genetic Systems™ rLAV EIA was estimated from the results of screening tests in random U.S. blood and plasma donors. Specificity was estimated by the following formula:

$$\frac{(\text{\# normal donor specimens} - \text{\# repeatedly reactive specimens}) \times 100}{(\text{\# normal donor specimens} - \text{\# repeatedly reactive specimens confirmed positive for antibodies to HIV-1})}$$

A total of 8,293 donor specimens were tested; 3 of these specimens were repeatedly reactive by the rLAV EIA. Of the repeatedly reactive specimens, none was confirmed to be positive for antibodies to HIV-1. Thus the Genetic Systems™ rLAV EIA has an estimated specificity of 99.96% (95% confidence interval: 99.91-100.00%) in serum and plasma.

Sensitivity Studies

Reactivity of Known HIV-1 Positive Specimens

The sensitivity of the Genetic Systems™ rLAV EIA was determined by testing serum and plasma samples from patients diagnosed as having AIDS (n = 201), ARC (n = 20), HIV-1 Asymptomatic (n=26) and from 498 individuals known to be HIV-1 antibody positive from U.S. (n=300) and non-U.S. locations (n=198) for whom the clinical status was unknown. The results of testing are shown in Table 3.

Table 3: Reactivity of HIV-1 Known Positive Specimens

Group	Results with Genetic Systems™ rLAV EIA			
	Number Tested	Non-Reactive	Initially Reactive	Repeatedly Reactive
AIDS	201 (100%)	0 (0.00%)	201 (100%)	201 (100%)
ARC	20 (100%)	0 (0.00%)	20 (100%)	20 (100%)
HIV-1 Asymptomatic	26 (100%)	0 (0.00%)	26 (100%)	26 (100%)
Known HIV-1 Positive U.S.	300 (100%)	0 (0.00%)	300 (100%)	300 (100%)
Known HIV-1 Positive Non-U.S.[a]	198 (100%)	0 (0.00%)	198 (100%)	198 (100%)
Total	**745 (100%)**	**0 (0.00%)**	**745 (100%)**	**745 (100%)**

[a]Non-U.S. locations included the following: Central African Republic (n=42); Nigeria (n=40); Zimbabwe (n=9); Australia (n=41); Thailand (n=41); Ghana (n=4); Nairobi (n=1); Sierra Leone (n=20).

All (100%, 745/745) of the known HIV-1 positive specimens were repeatedly reactive on the Genetic Systems™ rLAV EIA. All of the known positives were confirmed positive with a licensed HIV-1 Western blot.

Sensitivity of the Genetic Systems™ rLAV EIA was estimated from the results of testing 201 patients with AIDS. Studies demonstrated a positive test result in 201 of 201 patients for an estimated sensitivity of 100% (95% confidence interval: 99.75 to 100.00%). Additionally, sensitivity to antibody to HIV-1 was evaluated in subjects with ARC (n = 20), HIV-1 positive asymptomatic (n = 26), and known positive specimens from the U.S. (n = 300) and outside the U.S. (n= 198). In these studies, the rLAV EIA was positive with 544 of 544 subjects with a positive HIV-1 screening test which were additionally confirmed with a licensed HIV-1 Western blot.

Reactivity of Specimens from High-Risk Individuals

The results of testing for antibodies to HIV-1 in 500 sera/plasma specimens prospectively collected from individuals at high risk for HIV-1 infection are shown in Table 4. The numbers include 300 plasma specimens from prisoners, 100 sera from STD clinic patients, and 100 sera collected from patients at a hospital emergency room in a high HIV-1 prevalence area. All specimens were also screened with a licensed HIV-1 EIA. All specimens which were repeatedly reactive with the Genetic Systems™ rLAV EIA and/or the licensed HIV-1 EIA were tested with a licensed HIV-1 Western blot.

Table 4: Reactivity of Prospective Specimens from High-Risk Individuals

	Results with Genetic Systems™ rLAV EIA				Repeatedly Reactive Specimens
Group	Number Tested	Non-Reactive	Initially Reactive	Repeatedly Reactive	Positive by licensed HIV-1 Western blot
Prisoners	300 (100%)	288 (96.00%)	12 (4.00%)	12 (4.00%)	11
STD Clinic	100 (100%)	86 (86.00%)	14 (14.00%)	14 (14.00%)	14
E.R. Patients	100 (100%)	83 (83.00%)	17 (17.00%)	17 (17.00%)	16
Total	**500 (100%)**	**457 (91.40%)**	**43 (8.60%)**	**43 (8.60%)**	**41**

The Genetic Systems™ rLAV EIA detected 11/11 (100%) of the HIV-1 confirmed positive specimens from prisoners, 14/14 (100%) of the HIV-1 confirmed positives from STD patients, and 16/16 (100%) of the HIV-1 confirmed positives from hospital emergency room patients in a high prevalence area.

Reactivity with HIV-1 Seroconversion Panels

Sensitivity of the Genetic Systems™ rLAV EIA was also compared with previously licensed tests for antibody to HIV-1 using commercially available HIV-1 seroconversion panels and was equivalent or better, based on time of appearance of antibodies. The Genetic Systems™ rLAV EIA detected the presence of antibody to HIV-1 in specimens from thirty commercially available HIV-1 seroconversion panels as early as, or earlier than, a licensed HIV-1 EIA and HIV-1 Western blot. Of the thirty panels tested, Genetic Systems™ rLAV EIA detected 19 of the panels at an earlier bleed and 11 of the panels at the same bleed when compared to the licensed HIV-1 EIA. Twelve examples of the reactivity demonstrated by the rLAV EIA on seroconversion samples are shown in Table 5 below:

Table 5: Detection of Antibody to HIV-1 in Representative Seroconversion Panels

Panel	First Bleed Detected as Repeatedly Reactive/Positive					
	Genetic Systems™ rLAV EIA	Licensed Recombinant-based HIV-1 EIA	Licensed Viral Lysate-based HIV-1 EIAs			Licensed HIV-1 Western Blot*
			HIV-1 EIA #1*	HIV-1 EIA #2*	HIV-1 EIA #3	
PRB909	2	3	3	3	3	3
PRB914	1	1	1	4	4	4
PRB917	5	5	6	7	7	7
PRB919	2	2	2	UD**	UD**	2
PRB924	6	6	7	8	UD**	8
PRB925	5	5	5	6	6	UD**
PRB927	3	3	3	4	5	4
PRB930	4	4	4	UD**	UD**	UD**
PRB931	6	6	7	7	8	7
CPI-2	6	NA***	6	7	7	7
SVO-0231	C	C	E	E	F	D
SVO-0241	D	D	D	E	F	D

*Data from certificates of analysis from panel vendors.
**UD: Undetected as positive/reactive at any bleed
***NA: Not available

PERFORMANCE CHARACTERISTICS OF DRIED BLOOD SPOT TESTING
Specificity Studies

Reactivity of Dried Blood Spot Specimens from Random Blood Donors and Neonates

The results of testing dried blood spot specimens collected on filter paper from random blood donors and neonates with Genetic Systems™ rLAV EIA are summarized in Table 6 below. The data include 2,525 dried blood spot (DBS) specimens obtained from individuals at three geographically distinct locations.

Table 6: Detection of Antibodies to HIV-1 in Dried Blood Spot Specimens in Neonates and Random Blood Donors

Group	Results with Genetic Systems™ rLAV EIA			
	Number Tested	Non-Reactive	Initially Reactive	Repeatedly Reactive
Adults - Site 1	744 (100%)	744 (100%)	0 (0.00%)	0 (0.00%)
Adults - Site 2	780 (100%)	779 (99.87%)	1 (0.13%)	0 (0.00%)
Total Adults	**1524 (100%)**	**1523 (99.93%)**	**1 (0.07%)**	**0 (0.00%)**
Neonates - Site 3	1001 (100%)	996 (99.50%)	5 (0.50%)	4 (0.40%)

NA = Not Applicable

As shown in Table 6, 99.93% of the DBS from adult random donors were initially nonreactive, 0.07% were initially reactive and none (0.00%) was repeatedly reactive.

Of the 1,001 neonate DBS, 99.50% were initially nonreactive, 0.50% were initially reactive, and 0.40% were repeatedly reactive upon retesting. Four of 5 initially reactive specimens were repeatedly reactive upon retesting. None of the repeatedly reactive specimens was positive for antibodies to HIV-1 on an investigational HIV-1 Western blot.

Specificity of the Genetic Systems™ rLAV EIA when testing dried blood spot specimens (DBS) was estimated from neonates and adults, using the following formula:

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

A total of 1,524 dried blood spot specimens collected from random blood donors was tested; none of these specimens was repeatedly reactive by the rLAV EIA. Thus the Genetic Systems™ rLAV EIA has an estimated specificity of 100.00% (95% confidence interval: 99.97 to 100.00%) in testing adult DBS specimens.

A total of 1,001 DBS specimens collected from neonates was tested; four of these specimens were repeatedly reactive by the rLAV EIA. Of the repeatedly reactive specimens none was confirmed to be positive for antibodies to HIV-1. Thus the Genetic Systems™ rLAV EIA has an estimated specificity of 99.60% (95% confidence interval: 99.16 to 100.00%) in testing neonatal DBS specimens.

Sensitivity Studies

Reactivity of Known HIV-1 Positive DBS Specimens

The reactivity of known HIV-1 positive dried blood spots (DBS) on Genetic Systems™ rLAV EIA was determined by testing paired or simulated DBS from patients diagnosed as having AIDS (n=101), or ARC (n=20), HIV-1 positive asymptomatic (n=26), and from individuals known to be HIV-1 antibody positive from U.S. (n=50) and non-U.S. locations (n=50). Results of testing are shown in Table 7 below.

Table 7: Reactivity of Known HIV-1 Positive DBS Specimens

	Results with Genetic Systems™ rLAV EIA		
Group	Number Tested	Sample	Repeatedly Reactive
AIDS	101 (100%)	DBS (paired) Serum/Plasma	101 (100%) 101 (100%)
ARC	20 (100%)	DBS (paired) Serum/Plasma	20 (100%) 20 (100%)
HIV-1 Asymptomatic	26 (100%)	DBS (paired) Serum/Plasma	26 (100%) 26 (100%)
Known HIV-1 Positive U.S.	50 (100%)	DBS (simulated) Serum/Plasma	50 (100%) 50 (100%)
Known HIV-1 Positive Non-U.S.[a]	50 (100%)	DBS (simulated) Serum/Plasma	50 (100%) 50 (100%)
Total	**247 (100%)**	DBS Serum/Plasma	**247 (100%) 247 (100%)**

[a]Non-U.S. locations included the following: Central African Republic (8); Nigeria (7); Zimbabwe (7); Australia (8); Thailand (8); Ghana (4); Nairobi (1); Sierra Leone (7).

All (100%, 247/247) of the known HIV-1 positive DBS and serum or plasma samples were repeatedly reactive when tested on the Genetic Systems™ rLAV EIA. All of the serum or plasma samples were confirmed positive with a licensed HIV-1 Western blot.

Sensitivity of the Genetic Systems™ rLAV EIA was estimated from results of testing dried blood spot specimens collected from subjects with AIDS (n=101), ARC (n=20), HIV-1 positive asymptomatic (n=26), and known HIV-1 positive specimens from subjects in the U.S. (n=50) and outside the U.S. (n=50). In these studies rLAV EIA was positive with 247 of 247 subjects (100%) with a positive HIV-1 EIA screening test. In addition, the paired serum/plasma specimens were confirmed with a licensed Western blot. All (n=247) of these specimens were paired with serum/plasma and demonstrated 100% correlation for detection of HIV-1 antibody.

Reactivity of DBS Specimens from High-Risk Individuals

The results of testing 333 dried blood spots (DBS) for antibodies to HIV-1 with Genetic Systems™ rLAV EIA in specimens prospectively collected from individuals at high risk for HIV-1 infection are shown in Table 8. The numbers include 25 simulated DBS specimens (paired with serum or plasma) from patients at a hospital emergency room in a high HIV-1 prevalence area, 75 simulated DBS (paired with serum or plasma) from STD clinic patients, and 233 unpaired DBS collected from prostitutes (n=53), IV drug users (n=56), individuals in an alcohol treatment center (n=45), and homosexual/bisexual males (n=79). All DBS and simulated DBS specimens were also tested with a licensed HIV-1 EIA. Simulated dried blood spot specimens repeatedly reactive with the Genetic Systems™ rLAV EIA or the licensed HIV-1 EIA were compared to the sera/plasma result on a licensed HIV-1 Western blot. Unpaired dried blood spot specimens repeatedly reactive with the Genetic Systems™ rLAV EIA or the licensed EIA were tested on an investigational HIV-1 Western blot. All specimens that confirmed positive and were detected as repeatedly reactive by the licensed HIV-1 EIA were also detected as repeatedly reactive by the Genetic Systems™ rLAV EIA.

Table 8: Reactivity of Dried Blood Spot Specimens from High-Risk Individuals

	Results with Genetic Systems™ rLAV EIA			Repeatedly Reactive Specimens
Group	Number Tested	Sample	Repeatedly Reactive	Positive by a HIV-1 Western blot[a]
ER Patients from High Prevalence Area	25 (100%)	DBS (simulated) Serum/Plasma	2 (8.00%) 2 (8.00%)	2
STD Clinic Patients	75 (100%)	DBS (simulated) Serum/Plasma	9 (12.00%) 9 (12.00%)	9
Total	**100 (100%)**	**DBS (simulated) Serum/Plasma**	**11 (11.00%) 11 (11.00%)**	**11**
Prostitutes	53 (100%)	DBS (unpaired)	2 (3.77%)	2
IV Drug Users	56 (100%)	DBS (unpaired)	2 (3.57%)	1
Alcohol Treatment Patients	45 (100%)	DBS (unpaired)	2 (4.44%)	0
Homosexual/ Bisexual Males	79 (100%)	DBS (unpaired)	4 (5.06%)	3[b]
Total	**233 (100%)**	DBS (unpaired)	**10 (4.29%)**	**6**

[a]Serum/Plasma samples tested with a licensed HIV-1 Western blot. DBS tested with an investigational HIV-1 Western blot.
[b]Three of 4 unpaired DBS, repeatedly reactive on Genetic Systems™ rLAV EIA, were positive when tested on an investigational HIV-1 Western blot. One specimen GNS for testing on an investigational HIV-1 Western blot.

Sensitivity of the Genetic Systems™ rLAV EIA was also estimated from results of dried blood spot specimens from high risk individuals including E.R. patients from a high prevalence area (n=25), STD clinic patients (n=75), prostitutes (n=53), IV drug users (n=56), alcohol treatment patients (n=45), and homosexual/ bisexual males (n=79). In these studies the rLAV EIA was repeatedly reactive with 17 of 17 subjects with a repeatedly reactive HIV-1 EIA screening test which were additionally confirmed by immunoblot. Eleven of these specimens were paired with serum/plasma and demonstrated 100% correlation for detection of HIV-1 antibody.

Thus, the Genetic Systems™ rLAV EIA, when testing dried blood spot specimens, has an estimated sensitivity of 100% (95% confidence interval: 99.81% to 100%) in high-risk settings.

Reactivity of Simulated DBS Specimens from Seroconversion Panels

Testing of simulated dried blood spots prepared from 30 commercially available seroconversion panels resulted in sensitivity performance substantially equivalent to that observed with serum/plasma (Table 5). Of the 30 panels tested, Genetic Systems™ rLAV EIA detected 18 of the panels at an earlier bleed and 12 of the panels at the same bleed when compared to the licensed HIV-1 EIA.

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