Summary of Safety and Effectiveness

I. General Information

Reference No.: PMA BP 100064

Applicant: Bio-Rad Laboratories
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Generic Name: Human Immunodeficiency Virus (HIV) p24 antigen and antibodies to HIV Type 1 (HIV-1 group M and group O) and/or Type 2

Product Trade Name: GS HIV Combo Ag/Ab EIA

Date of Panel Recommendation: Not Applicable

Date of Notice of Approval to the Applicant:

II. Indications For Use

The GS HIV Combo Ag/Ab EIA is an enzyme immunoassay kit for the simultaneous qualitative detection of Human Immunodeficiency Virus (HIV) p24 antigen and antibodies to HIV Type 1 (HIV-1 groups M and O) and HIV Type 2 (HIV-2) in human serum or plasma. This kit is intended as an aid in the diagnosis of HIV-1 and/or HIV-2 infection, including acute or primary HIV-1 infection. The assay may also be used as an aid in the diagnosis of HIV-1 and/or HIV-2 infection in pediatric subjects (i.e., children as young as 2 years of age). The GS HIV Combo Ag/Ab EIA is intended for manual use and for use with the Bio-Rad EVOLIS™ Automated Microplate System.

Results from the GS HIV Combo Ag/Ab EIA cannot be used to distinguish between the presence of HIV p24 antigen, HIV-1 antibody, or HIV-2 antibody in a sample.

The GS HIV Combo Ag/Ab EIA is not intended for routine use in screening blood or plasma donors, as the effectiveness of this test for use in the screening of these donors has not been established. However, in urgent situations where traditional licensed blood donor screening tests are unavailable or their use is impractical, this assay can be used as a blood donor screening assay.

III. Device Description

The GS HIV Combo Ag/Ab EIA is an enzyme immunoassay based on the principle of the sandwich technique for the qualitative detection of HIV-1 p24 antigen and detection of envelope antibodies associated with HIV-1 and/or HIV-2 virus in human serum or plasma. The solid phase is coated with:

- Monoclonal antibodies against HIV-1 p24 antigen
- HIV antigens: HIV-1 gp160 recombinant protein, a synthetic peptide mimicking a totally artificial (i.e. encoded by no existing virus) HIV-1 group O-specific epitope, and a peptide mimicking the immunodominant epitope of the HIV-2 envelope protein.

The conjugates are based upon the use of:

- Biotinylated polyclonal antibodies to HIV p24 Ag (Conjugate 1)
- Peroxidase-conjugated streptavidin and peroxidase-conjugated HIV-1 antigens (gp41 and gp36 peptides mimicking the immunodominant epitopes of the HIV-1 and HIV-2 envelope glycoproteins, and the same synthetic peptide mimicking a totally artificial HIV-1 group O-specific epitope used for the solid phase) (Conjugate 2)

During the assay procedure, Conjugate 1 (biotinylated polyclonal antibody to HIV p24 Ag) is added to the microplate wells, followed by the addition of samples to be assayed, as well as controls and a calibrator. If present, HIV p24 antigen binds to the monoclonal antibody on the solid phase and also binds to the Conjugate 1. HIV-1 and/or HIV-2 antibodies, if present, bind to the antigens immobilized on the solid phase. The addition of Conjugate 1 and sample is validated through a color change from yellow-green to blue. After incubation, excess sample is removed by a wash step. Next, Conjugate 2 is added. Peroxidase-labeled streptavidin reacts with biotinylated Ab-Ag-Ab complexes; peroxidase-labeled HIV-1 and HIV-2 antigens bind to the IgG, IgM, or IgA antibodies captured on the solid phase. After incubation, unbound Conjugate 2 is removed by washing. Working TMB Solution is added to the plate and allowed to incubate. A blue or blue-green color develops in proportion to the amount of HIV antibody and/or antigen present in the sample. Color development is stopped by the addition of acid, which changes the blue-green color to yellow. The optical absorbances of specimens, controls, and the calibrator are determined spectrophotometrically at a wavelength of 450 nm with a 615 to 630 nm reference.

Components of the GS HIV Combo Ag/Ab EIA are listed below:

1. Microwell Strip Plates: Microwell plate coated with monoclonal antibodies to HIV p24 (mouse) and HIV-1 and HIV-2 antigens. Preservative: ProClin 300.
2. Wash Solution: Contains Sodium chloride and Tween 20™.
3. Negative Control: Human serum/plasma; negative for antibodies to HIV and HCV; negative for HBsAg and HIV-1 Ag. Preservatives: 0.005% Gentamicin sulfate and 0.16% ProClin 950.
4. HIV-1 Ab Positive Control: Human HIV-1 antibody in human serum/plasma; negative for HIV-1 Ag, HBsAg and anti-HCV antibodies. Preservative: 0.005% Gentamicin sulfate and 0.16% ProClin 950.
5. HIV-2/O Ab Positive Control: Mouse monoclonal antibody to HIV-2 and rabbit antibody to HIV-1 Group O in human serum/plasma; negative for HBsAg and anti-HCV antibodies. Preservatives: 0.005% Gentamicin sulfate and 0.16% ProClin 950.
6. HIV Ag Positive Control: Purified HIV-1 viral lysate inactivated with heat and a chaotropic agent, in synthetic diluent. Preservative: 0.1%ProClin 300.
7. Cutoff Calibrator: Human serum/plasma; negative for antibodies to HIV and HCV; negative for HBsAg and HIV-1 Ag. Preservatives: 0.005% Gentamicin sulfate and 0.16% ProClin 950.
8. Conjugate 1: Biotinylated polyclonal antibodies (sheep) to HIV p24 Ag with yellow-green dye. Preservatives: 0.5% ProClin 300 and 0.005% Gentamicin sulfate.
9. Conjugate 2: Lyophilized, peroxidase-labeled Streptavidin and peroxidase-labeled HIV-1 and HIV-2 antigens. Preservative: 0.5% ProClin 300 and 0.005% Gentamicin sulfate.
10. Conjugate 2 Diluent: Buffer with protein stabilizers and red dye. Preservatives: 0.5% ProClin 300 and 0.005% Gentamicin sulfate.
11. Substrate Buffer: Hydrogen peroxide and Citric acid/sodium acetate buffer

12 Chromogen: Tetramethylbenzidine (TMB)

13. Stopping Solution: 1N H_2SO_4 (Sulfuric acid)
14. Plate Sealers: Clear plastic sealers

IV. Warnings, Precautions, and Contraindications

For *in vitro* diagnostic use only.

Warnings and precautions relating to the GS HIV Combo Ag/Ab EIA are contained in the product labeling.

There are no known contraindications.

V. Alternative Practices and Procedures

The detection of antibodies to HIV-1 and HIV-2 in humans is primarily performed using laboratory-based assays for serum, plasma, oral fluid, or urine. The majority of these tests use principles similar to that of the GS HIV Combo Ag/Ab EIA, utilizing peptides, recombinant antigens, isolated proteins, or viral lysate immobilized onto a solid phase support to capture antibodies in a patient sample. For early diagnosis in the acute phase, HIV-1 antigen can also be detected by EIA testing, or nucleic assay amplification testing can be used. Test results are to be used in conjunction with clinical symptoms in the diagnosis of infection with HIV.

VI. Marketing History

The GS HIV Combo Ag/Ab EIA has not been marketed previously.

VII. Summary of Preclinical Studies

A. Reactivity with Sensitivity and Seroconversion Panels

HIV-1 p24 Antigen Analytical Sensitivity

The GS HIV Combo Ag/Ab EIA was designed to have an analytical sensitivity of < 50 pg/ml for HIV-1 p24 antigen on a panel derived from the Agence Française de sécurité sanitaire des produits de santé (AFSSAPS) and < 2 IU/mL on the WHO HIV international standard NIBSC 90/636. In an internal study the results demonstrated an antigen sensitivity of 14.78 pg/mL (range of 13.22-15.89 pg/mL) on the AFSSAPS standard and an antigen sensitivity of 0.65 IU/mL (range of 0.40-1.05 IU/mL) on the WHO standard.

HIV-1 Antigen Detection in Culture Supernatants

Fifty-three (53) HIV-1 culture supernatants were tested on the GS HIV Combo Ag/Ab EIA. These supernatants were from subtypes A (N=10), B (N=10), C (N=9), D (N=4), AE (N=10), F (N=2), G (N=2), H (N=1), J (N=2), N (N=1), and O (N=2**)**. Due to the limited volumes of the HIV-1 culture supernatant samples, all samples were diluted before testing. Of the 53 diluted HIV-1 culture supernatant samples tested with the GS HIV Combo Ag/Ab EIA, 100% (53/53) were reactive.

HIV-1 Seroconversion Panels

Thirty (30) commercially available seroconversion panels (228 total members) were tested on the GS HIV Combo Ag/Ab EIA and an FDA licensed HIV-1/HIV-2 EIA. The GS HIV Combo Ag/Ab EIA detected reactive bleeds in 96.7% (29/30) of the seroconversion panels compared to 83.3% (25/30) detected by an FDA licensed HIV-1/HIV-2 EIA and 23.3% (7/30) detected by the HIV-1 Western blot. Reactivity in 4 of the panels was detected only by the GS HIV Combo Ag/Ab EIA. Of the 25 panels that were reactive by both GS HIV Combo Ag/Ab EIA and the HIV-1/HIV-2 EIA, the first reactive bleed occurred earlier on the GS HIV Combo Ag/Ab EIA in 84.0% (21/25) of the panels. Sixteen percent (16.0%; 4/25) of the panels were detected at the same bleed by both GS HIV Combo Ag/Ab EIA and the HIV-1/HIV-2 EIA. Compared to the FDA licensed third generation HIV-1/HIV-2 EIA, the GS HIV Combo Ag/Ab EIA reduced the time to detection of HIV (i.e. window period), with an overall range of 0 to 20 days for the 30 seroconversion panels tested in this study. Therefore, the GS HIV Combo Ag/Ab EIA demonstrated a greater capability of detecting acute and primary HIV infection than either a third generation HIV-1/HIV-2 EIA or an HIV-1 Western blot.

Twenty (20) of the 30 HIV-1 seroconversion panels (N = 153 samples) that were tested manually were also tested using the EVOLIS™ Automated Microplate System. The GS HIV Combo Ag/Ab EIA manual assay results and the EVOLIS™ results were equivalent in reactivity for 98.7% (151/153) of the seroconversion panel specimens tested. Two (2) seroconversion panel members were reactive with the GS HIV Combo Ag/Ab EIA by EVOLIS™ testing one bleed earlier than testing by the manual method.

HIV-1 Group M Subtype Infectivity Panel

A commercially available 15 member HIV-1 Group M Subtype Infectivity Panel [subtypes A, A1, B, C, D, F1, F2, G, CRF01, CRF02, CRF05, CRF06, CRF09, CRF11, and CRF13] was tested with the GS HIV Combo Ag/Ab EIA. This subtype panel was repeatedly reactive in 100% (15/15) of the members on the GS HIV Combo Ag/Ab EIA.

Low Titer Panel

A commercially available HIV-1 Low Titer Panel consists of 15 frozen plasma members with HIV-1 antibody responses near the sensitivity limit of anti-HIV screening tests. One anti-HIV-1 negative specimen is included in the panel as a negative control. Criteria for inclusion in the panel were signal-to-cutoff ratio (S/CO) within the dynamic range of the assay on at least one FDA licensed anti-HIV screening test and one of the following: a minimally positive, indeterminate, or negative Western blot, plus detectable HIV RNA.

Reactivity of the GS HIV Combo Ag/Ab EIA with the HIV-1 Low Titer Panel (N =15) was compared to an FDA licensed HIV-1/HIV-2 EIA and a licensed HIV-1 Western blot. The GS HIV Combo Ag/Ab EIA and the HIV-1/HIV-2 EIA were equivalent in 100% (14/14) of the reactive HIV-1 Low Titer Panel samples. Only 50% (7/14) of the reactive HIV-1 Low Titer panel members were positive on the HIV-1 Western blot.

Ten (10) members of the Low Titer Panel were also tested using the EVOLIS™ Automated Microplate System. The GS HIV Combo Ag/Ab EIA tested with the EVOLIS™ was equivalent in reactivity in 100% (10/10) of the samples in the HIV-1 Low Titer Panel compared to the manual method.

HIV-1 Incidence / Prevalence Panel

A commercially available HIV-1 Incidence/Prevalence Panel consists of 15 members (plasma) from different donors. Seven members are characterized as incident and eight as prevalent, based on consensus results from nine tests using five different methods. Specimens are undiluted aliquots from plasma units collected between 1996 and 1998, from HIV positive deferred donors in the United States whose dates of infection and seroconversion are unknown.

An HIV-1 Incidence / Prevalence Panel (N=15) was tested on the GS HIV Combo Ag/Ab EIA and on a licensed HIV-1/HIV-2 EIA, and compared to the HIV-1 Western blot historical data provided in the Certificate of Analysis (C of A). The GS HIV Combo Ag/Ab EIA, the licensed HIV-1/HIV-2 EIA and the HIV-1 Western blot were equivalent in 100% (15/15) of the HIV-1 Low Titer Panel samples.

The HIV-1 Incidence/ Prevalence Panel (N = 15) members were also tested using the EVOLIS™ Automated Microplate System. The GS HIV Combo Ag/Ab EIA tested with the EVOLIS™ was equivalent in reactivity in 100% (15/15) of the samples in the HIV-1 Incidence/Prevalence Panel when compared to the manual method.

B. Interfering Substances

The GS HIV Combo Ag/Ab EIA was evaluated to determine if there were any effects on assay performance when tested with specimens containing potentially interfering substances. Studies included HIV negative samples and those spiked with both low and high levels of HIV-1 antigen, HIV-1 antibody, and HIV-2 antibody. In these studies the samples were spiked with the substances at two levels, including the upper levels listed here that were tested in 7 samples each.

Hemolyzed: 500 mg/dL of hemoglobin
Lipemic: 1000 mg/dL of triglycerides
Icteric: 20 mg/dL of bilirubin
Proteinemic: 12 g/dL of protein

The potential interferents at the levels tested above did not produce a change in clinical interpretation. Any changes in mean S/CO that were observed for the high and low positive

samples and for the negative samples spiked with these potential interferents were not significant**.**

C. Anticoagulants

Specimen collection tube studies support the use of the following tube types and anticoagulants, including those in both glass and plastic tubes, in testing with the GS HIV Combo Ag/Ab EIA: serum tubes, serum separator tubes (SSTs) with and without activator, potassium EDTA, sodium citrate, sodium and lithium heparin, and plasma separator tubes (PSTs).

D. Unrelated Medical Conditions

The reactivity rate of the GS HIV Combo Ag/Ab EIA was determined in samples from individuals with medical conditions unrelated to HIV infection. Samples that were repeatedly reactive with the GS HIV Combo Ag/Ab EIA were tested with a licensed HIV-1/HIV-2 EIA, an approved HIV-1/HIV-2 differentiation test, an HIV-1 p24 Ag EIA, and a licensed HIV-1 Western blot. Results are shown in Table 1.

Table 1
Unrelated Medical Conditions

Unrelated Medical Condition	N	GS HIV Combo Ag/Ab EIA Results	
		Non Reactive	Repeatedly Reactive
Immunological disease [a]	20	20	0
Anti - EBV	20	20	0
Anti - HCV	20	20	0
Anti – HTLV	20	20	0
HBsAg +	20	20	0
Vaccinia Vaccine	20	20	0
Pre-Influenza Vaccine	20	20	0
Post-Influenza Vaccine	20	18	2 [b]
Candida Infection	20	19	1 [c]
Hemodialysis	20	20	0
Hemophilia	20	20	0
Multiple Transfusions	20	20	0
Multiparous	20	20	0
RF +	20	20	0
Cord Blood	20	20	0
Total	**300**	**297**	**3**

[a] Includes: 6 Scleroderma, 5 Systemic Lupus Erythematosus (SLE), 5 ANA positive, 2 Sjögren's Syndrome (SS) and 2 Mixed Connective Tissue Disease (MCTD) samples.
[b] Twenty (20) additional post influenza vaccine samples were tested and all 20 were nonreactive.
[c] This sample was HIV reactive and undifferentiated on the HIV-1/HIV-2 differentiation test.

Three (3) specimens from individuals with unrelated medical conditions were repeatedly reactive with the GS HIV Combo Ag/Ab EIA. These 3 specimens were also repeatedly reactive on the licensed HIV-1/HIV-2 EIA. Of the 3 repeatedly reactive samples, 2 were from post-influenza vaccine subjects and 1 was from an individual positive for Candida infection. All 3 specimens were negative for HIV-1 antibodies on an HIV-1 Western blot and an HIV-1 p24 Ag EIA. The two specimens from post-influenza vaccine recipients were nonreactive on the HIV-1/HIV-2 differentiation test and the Candida infection specimen was HIV reactive (undifferentiated) on the same assay. The pre- and post-influenza vaccine specimens were from different individuals.

E. Dose Effect

The GS HIV Combo Ag/Ab EIA did not exhibit a high-dose hook effect in patient samples with high levels of HIV antibodies (i.e., samples that were reactive at $>10^6$ dilution).

F. Stability Studies

1. Kit Stability

A functional stability study of the GS HIV Combo Ag/Ab EIA test kit demonstrates that kits which are stored at 2-8°C are stable for the intended shelf-life of the kits. The real-time studies include testing on three kit lots, at multiple time points throughout the shelf life of the kits. The expiration of the assembled kit is based on the component with the shortest dating period.

2. Interchangeability of Common Reagents

The GS HIV Combo Ag/Ab EIA contains four common reagents that may be used interchangeably with the same components in other lots of the kit: Wash Solution Concentrate, Chromogen, Substrate Buffer, and Stopping Solution. Matrix studies performed with the GS HIV Combo Ag/Ab EIA have evaluated different lots of each of these components in the kit, and demonstrated equivalent results. Therefore, any lot number of these reagents may be used with this assay provided they are not used beyond their labeled expiration date.

G. Microbiology Studies

Antimicrobial preservatives have been added to the components in the GS HIV Combo Ag/Ab EIA to protect the product from degradation and performance failure due to the presence of microbial contamination. Preservative effectiveness studies have been conducted in accordance with the protocol specified in the United States Pharmacopoeia (Microbiological Tests, <51> Antimicrobial Preservatives/Effectiveness) to assess the efficacy of these preservatives in suppressing microbial growth. These studies have demonstrated that the antimicrobial agents are present in concentrations required to inhibit the growth of adventitious agents.

A Microbial Challenge study has been performed to evaluate the functional stability of the GS HIV Combo Ag/Ab EIA components in the presence of microbial organisms. One set of GS HIV Combo Ag/Ab EIA components that were inoculated with microorganisms was tested in comparison to a reference second set of GS HIV Combo Ag/Ab EIA components that had not been inoculated. A variety of organisms from the environment were used in this challenge study. Each kit was stored at the recommended product storage of 2-8°C after inoculation and tested at multiple time points throughout kit expiration. These studies demonstrate that the functionality of the product is not impaired and the reagents are stable for the stated shelf life when microbial contamination is present.

H. Reproducibility and Precision Testing

A panel of 19 specimens was used for determining the reproducibility and precision of the GS HIV Combo Ag/Ab EIA. The 19-member reproducibility panel included 8 serum members (6 positive and 2 negative), 6 plasma (EDTA) members (4 positive and 2 negative) and the 5 GS HIV Combo Ag/Ab EIA kit controls/calibrator. The composition of the panel was as follows:

#	Panel Member Composition
1	HIV-1 Ab Positive Control
2	HIV2/O Ab Positive Control
3	HIV Ag Positive Control
4	Cutoff Calibrator
5	Negative Control
6	HIV-1 Positive (Serum)
7	HIV-1 Low Positive (Serum)
8	HIV-1 High Negative (Serum)
9	HIV-1 Low Positive (EDTA)
10	HIV-1 Group O Low Positive (Serum)
11	HIV-1 Group O Low Positive (EDTA)
12	HIV-2 Positive (Serum)
13	HIV-2 Low Positive (Serum)
14	HIV-2 Low Positive (EDTA)
15	HIV-2 High Negative (EDTA)
16	HIV-1 Ag Low Positive (Serum)
17	HIV-1 Ag Low Positive (EDTA)
18	Negative (Serum)
19	Negative (EDTA)

Reproducibility

Reproducibility of the GS HIV Combo Ag/Ab EIA was determined for both manual microplate and EVOLIS microplate processing. Three (3) lots of the GS HIV Combo Ag/Ab EIA were used in the evaluation, and testing was performed at three sites. Each panel member was tested in triplicate (x3) on 1 run per day for 5 days on 1 lot of the GS HIV Combo Ag/Ab EIA at each site (15 replicates per member per lot/site). The data were analyzed for within-run and between-day reproducibility according to the principles described in the Clinical Laboratory Standards Institute (CLSI) EP15-A2. The standard deviation (SD) and percent coefficient of variation (% CV) were calculated. Results were analyzed for both manual (Table 2) and EVOLIS™ testing (Table 3) separately.

Table 2
Reproducibility Results - Manual Testing
By Signal-to-Cutoff Ratio (S/CO), N = 15 at each site

Panel Member	Grand Mean (S/CO)	Site 1						Site 2						Site 3						Total Overall	
		Within Run		Between Day		Overall		Within Run		Between Day		Overall		Within Run		Between Day		Overall			
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	7.55	0.426	6.4	0.186	2.8	0.465	7.0	0.477	5.9	0.982	12.2	1.092	13.6	0.738	9.2	0.415	5.2	0.847	10.6	1.126	14.9
2	4.99	0.117	1.9	0.574	9.5	0.586	9.6	0.199	4.4	0.727	16.1	0.754	16.7	0.180	4.1	0.273	6.2	0.327	7.4	1.072	21.5
3	7.60	0.330	4.0	0.228	2.8	0.401	4.8	0.352	4.2	0.533	6.3	0.639	7.6	0.551	9.0	0.605	9.9	0.819	13.4	1.425	18.7
4	0.19	0.013	5.9	0.026	11.9	0.029	13.3	0.007	3.6	0.017	8.8	0.018	9.5	0.008	4.5	0.015	8.8	0.017	9.9	0.032	16.5
5	0.19	0.010	4.7	0.026	12.2	0.027	13.1	0.012	6.3	0.026	13.4	0.028	14.8	0.008	4.5	0.005	3.1	0.010	5.5	0.027	13.8
6	2.63	0.084	3.7	0.077	3.4	0.114	5.1	0.303	10.4	0.525	17.9	0.606	20.7	0.153	5.6	0.176	6.5	0.234	8.6	0.489	18.6
7	1.10	0.029	2.8	0.049	4.8	0.057	5.6	0.046	3.8	0.238	19.5	0.242	19.8	0.041	3.8	0.099	9.4	0.107	10.1	0.178	16.1
8	0.72	0.015	2.3	0.025	3.8	0.029	4.4	0.033	4.1	0.150	18.9	0.153	19.3	0.017	2.4	0.041	5.9	0.044	6.4	0.109	15.2
9	1.08	0.047	4.7	0.029	2.8	0.055	5.5	0.186	15.8	0.142	12.0	0.234	19.8	0.084	8.1	0.064	6.1	0.105	10.1	0.169	15.7
10	0.84	0.033	4.0	0.065	7.9	0.072	8.9	0.059	8.1	0.089	12.2	0.107	14.6	0.028	2.9	0.089	9.2	0.093	9.6	0.146	17.5
11	0.80	0.029	3.5	0.048	5.8	0.056	6.8	0.051	6.4	0.142	17.9	0.151	19.0	0.030	3.9	0.048	6.3	0.057	7.4	0.096	12.0
12	1.75	0.164	7.2	0.159	7.0	0.228	10.0	0.064	5.2	0.105	8.6	0.123	10.1	0.075	4.2	0.039	2.2	0.084	4.8	0.546	31.2
13	1.04	0.069	6.2	0.082	7.4	0.107	9.6	0.056	6.7	0.111	13.4	0.124	15.0	0.047	4.0	0.081	6.8	0.093	7.9	0.210	20.2
14	0.86	0.084	7.9	0.131	12.4	0.155	14.7	0.092	13.4	0.037	5.4	0.099	14.4	0.046	5.4	0.042	4.9	0.062	7.3	0.213	24.6
15	0.57	0.034	5.1	0.052	7.8	0.062	9.4	0.016	3.7	0.061	14.1	0.063	14.6	0.029	4.6	0.024	3.8	0.037	6.0	0.131	23.0
16	1.16	0.041	3.4	0.087	7.3	0.096	8.1	0.048	3.5	0.053	3.9	0.071	5.2	0.043	4.6	0.046	5.0	0.063	6.8	0.228	19.7
17	1.24	0.062	4.8	0.076	5.8	0.098	7.5	0.106	7.1	0.092	6.1	0.141	9.4	0.049	5.3	0.029	3.2	0.057	6.2	0.312	25.1
18	0.23	0.011	4.2	0.057	21.6	0.059	22.0	0.017	6.6	0.007	2.8	0.018	7.2	0.009	5.2	0.021	12.3	0.023	13.4	0.062	27.1
19	0.18	0.016	6.9	0.031	13.7	0.034	15.3	0.016	8.7	0.017	9.3	0.023	12.7	0.009	7.2	0.013	10.3	0.016	12.6	0.053	29.7

[1] Negative variances were rounded to zero, per statistical convention.

Table 3
Reproducibility Results – EVOLIS™ Testing
By Signal-to-Cutoff Ratio (S/CO), N = 15 at each site

Panel Member	Grand Mean (S/CO)	Site 1						Site 2						Site 3						Total Overall	
		Within Run		Between Day		Overall		Within Run		Between Day		Overall		Within Run		Between Day		Overall			
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	9.16	0.585	6.7	0.137	1.6	0.601	6.9	0.382	4.0	0.145	1.5	0.408	4.3	0.510	5.5	0.000[1]	0.0	0.510	5.5	0.665	7.3
2	5.67	0.222	3.2	0.507	7.2	0.554	7.9	0.509	9.6	0.451	8.5	0.680	12.8	0.138	3.0	0.349	7.5	0.376	8.0	1.311	23.1
3	8.39	0.209	2.3	0.177	2.0	0.274	3.0	0.129	1.5	0.223	2.6	0.257	3.0	0.169	2.2	0.102	1.3	0.197	2.6	0.730	8.7
4	0.20	0.006	3.9	0.009	5.7	0.011	6.8	0.008	3.6	0.010	4.7	0.013	6.0	0.011	5.0	0.013	6.2	0.017	8.0	0.036	18.2
5	0.20	0.012	6.2	0.004	2.2	0.012	6.6	0.007	3.5	0.011	5.5	0.013	6.5	0.014	6.5	0.022	10.3	0.026	12.2	0.023	11.3
6	3.16	0.103	3.6	0.167	5.9	0.196	6.9	0.123	3.5	0.175	5.0	0.214	6.2	0.195	6.2	0.061	1.9	0.205	6.5	0.366	11.6
7	1.29	0.014	1.2	0.076	6.4	0.077	6.5	0.043	3.0	0.033	2.3	0.054	3.8	0.034	2.7	0.051	4.0	0.061	4.8	0.133	10.3
8	0.82	0.019	2.5	0.055	7.2	0.058	7.6	0.019	2.1	0.022	2.5	0.029	3.3	0.018	2.2	0.037	4.5	0.041	5.0	0.077	9.4
9	1.32	0.087	6.9	0.062	4.9	0.106	8.5	0.073	5.1	0.060	4.2	0.094	6.5	0.090	7.2	0.048	3.9	0.102	8.1	0.143	10.8
10	1.17	0.071	5.6	0.069	5.5	0.099	7.8	0.130	11.0	0.194	16.4	0.233	19.7	0.074	7.0	0.066	6.3	0.099	9.4	0.179	15.3
11	0.99	0.048	4.3	0.028	2.5	0.055	5.0	0.064	6.0	0.144	13.6	0.158	14.9	0.017	2.2	0.060	7.6	0.063	7.9	0.195	19.7
12	2.46	0.378	11.6	0.118	3.6	0.396	12.1	0.135	6.2	0.333	15.4	0.360	16.6	0.100	5.2	0.103	5.3	0.143	7.4	0.770	31.3
13	1.40	0.147	9.3	0.190	12.0	0.240	15.2	0.049	3.7	0.229	17.6	0.234	18.0	0.042	3.1	0.123	9.3	0.130	9.8	0.245	17.5
14	1.18	0.189	13.2	0.000[1]	0.0	0.189	13.2	0.159	13.5	0.130	11.1	0.206	17.5	0.042	4.5	0.055	5.8	0.069	7.4	0.292	24.7
15	0.78	0.085	10.6	0.117	14.6	0.144	18.1	0.081	9.6	0.102	12.1	0.130	15.4	0.032	4.6	0.051	7.3	0.060	8.6	0.129	16.6
16	1.19	0.049	4.2	0.049	4.2	0.069	5.9	0.046	3.5	0.052	3.9	0.070	5.3	0.027	2.5	0.066	6.1	0.072	6.6	0.139	11.7
17	1.31	0.044	3.4	0.027	2.1	0.051	4.0	0.034	2.3	0.077	5.2	0.084	5.7	0.067	5.9	0.000[1]	0.0	0.067	5.9	0.186	14.2
18	0.22	0.011	5.7	0.007	3.6	0.012	6.7	0.012	5.0	0.014	6.0	0.018	7.8	0.014	5.5	0.034	13.4	0.037	14.5	0.042	18.9
19	0.19	0.006	3.6	0.013	8.0	0.015	8.8	0.008	4.2	0.008	4.2	0.011	5.9	0.024	11.4	0.021	10.1	0.032	15.2	0.031	16.3

[1] Negative variances were rounded to zero, per statistical convention.

Precision

A precision study was performed with the GS HIV Combo Ag/Ab EIA on the EVOLIS™ Automated Microplate System using a panel of 19 samples that were tested in duplicate, twice a day, for 20 days. The results are summarized in Table 4.

Table 4
Precision Results - EVOLIS Testing
By Signal-to-Cutoff Ratio (S/CO), N = 80

Panel Member	Mean S/CO	Within Run		Between Run		Between Day		Total[1]	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	9.80	0.385	3.9	0.277	2.8	0.161	1.6	0.501	5.1
2	7.46	0.566	7.6	0.123	1.6	0.350	4.7	0.677	9.1
3	8.25	0.203	2.5	0.229	2.8	0.095	1.2	0.321	3.9
4	0.18	0.010	5.2	0.009	5.1	0.004	2.0	0.014	7.6
5	0.20	0.016	7.9	0.009	4.4	0.005	2.6	0.019	9.4
6	3.36	0.137	4.1	0.165	4.9	0.127	3.8	0.249	7.4
7	1.48	0.037	2.5	0.090	6.1	0.063	4.3	0.116	7.8
8	1.01	0.069	6.8	0.035	3.4	0.035	3.5	0.085	8.4
9	1.65	0.094	5.7	0.060	3.7	0.083	5.0	0.139	8.5
10	1.42	0.158	11.2	0.104	7.3	0.081	5.7	0.206	14.5
11	1.32	0.096	7.2	0.093	7.1	0.108	8.2	0.172	13.0
12	2.80	0.389	13.9	0.126	4.5	0.134	4.8	0.431	15.4
13	1.66	0.234	14.1	0.000[2]	0.0	0.141	8.5	0.273	16.5
14	1.34	0.164	12.3	0.108	8.1	0.101	7.6	0.221	16.5
15	0.80	0.076	9.4	0.056	7.0	0.075	9.4	0.121	15.0
16	1.30	0.050	3.9	0.035	2.7	0.038	2.9	0.072	5.5
17	1.45	0.058	4.0	0.046	3.2	0.044	3.1	0.086	6.0
18	0.25	0.021	8.3	0.013	5.0	0.012	4.7	0.027	10.8
19	0.20	0.024	12.1	0.007	3.6	0.004	2.1	0.025	12.8

[1] Total: Total variability of the assay performance includes within run, between run and between day.
[2] Negative variances were rounded to zero, per statistical convention.

I. Animal studies

No animal studies were performed during evaluation of the GS HIV Combo Ag/Ab EIA.

VIII. Summary of Clinical Studies

A. Specificity

Low Risk Populations

Seven thousand samples (6000 serum and 1000 plasma) from populations at low risk for HIV infection were tested with GS HIV Combo Ag/Ab EIA. Repeatedly reactive (RR) samples were tested by an FDA licensed HIV-1 Western blot and an FDA-approved HIV-1/HIV-2 test that differentiates HIV-1 from HIV-2. Results are presented in Table 5.

Table 5
Reactivity in Low Risk Populations

Low Risk Population	Number Tested	GS HIV Combo Ag/Ab EIA			Repeatedly Reactive Specimens	
		Non-Reactive	Initially Reactive	Repeatedly Reactive	HIV-1 Western Blot Positive	HIV-2 Reactive*
Health Insurance Applicants	2000	1993 (99.65%)	7 (0.35%)	6 (0.30%)	2 (0.10%)	0 (0.00%)
Normal Blood Donors	2000	1998 (99.90%)	2 (0.10%)	0 (0.00%)	NT	NT
Low Risk Population - Other	1000	996 (99.60%)	4 (0.40%)	2 (0.20%)	0 (0.00%)	0 (0.00%)
Pregnant Women	1000	997 (99.70%)	3 (0.30%)	2 (0.20%)	1 (0.10%)	0 (0.00%)
Military Recruits - Frozen	498	494 (99.20%)	4 (0.80%)	3 (0.60%)	1 (0.20%)	0 (0.00%)
Military Recruits - Fresh	502	502 (100%)	0 (0.00%)	0 (0.00%)	NT	NT
Total	7000	6980 (99.71%)	20 (0.29%)	13 (0.19%)	4 (0.06%)	0 (0.00%)

* Based on HIV differentiation test results
NT = Not Tested

As shown in Table 5, 99.71% (6980/7000) of the low risk populations were initially nonreactive, 0.29% (20/7000) were initially reactive, and 0.19% (13/7000) were repeatedly reactive. Of the 13 repeatedly reactive specimens, 4 specimens were confirmed positive for antibodies to HIV-1 by Western blot and none were reactive for HIV-2 on the HIV-2 differentiation test. Of the 9 repeatedly reactive samples that were not confirmed by HIV-1 Western blot, 8 were also negative by HIV-1 Ag testing. The specificity of the GS HIV Combo Ag/Ab EIA in the low risk populations in this study was 99.87% (6987/6996) with a 95% confidence interval of 99.76% - 99.93%.

The results for the combined samples from low risk populations tested with the GS HIV Combo Ag/Ab EIA compared to results of testing the same samples with a licensed HIV-1/HIV-2 EIA are shown in Table 6.

Table 6
Combined Low Risk Populations – Assay Comparison Summary

GS HIV Combo Ag/Ab EIA Result	Licensed HIV-1/HIV-2 Result	N	HIV-1 Western Blot			HIV Differentiation Test		
			Pos	Ind	Neg	HIV-1 Reactive	HIV-2 Reactive	NR
Repeatedly Reactive	Repeatedly Reactive	7[a]	4	2	1	4	0	3
Repeatedly Reactive	Non-Reactive	6[b]	0	1	5	0	0	6
Non-Reactive	Repeatedly Reactive	1[c]	0	0	1	0	0	1
Non-Reactive	Non-Reactive	6986	NT	NT	NT	NT	NT	NT
Total		7000	**4**	**3**	**7**	**4**	**0**	**10**

[a] Of the 3 samples that were nonreactive on the HIV differentiation test, 2 were negative for HIV antigen with an HIV-1 Ag EIA and one was not available for testing
[b] HIV-1 Ag testing with an HIV-1 Ag EIA was negative for 5 of these samples; and one sample was negative with an HIV-1 RNA assay
[c] Faint (+/-) gp160 band on the HIV-1 Western Blot.
NT= Not Tested

Of the 7000 samples from the low risk populations that were tested manually with the GS HIV Combo Ag/Ab EIA, 3002 of the same samples were tested with the GS HIV Combo Ag/Ab EIA on the EVOLIS™ Automated Microplate System. The results by EVOLIS™ testing were equivalent in 99.97% (3001/3002) of the samples from low risk individuals compared to the manual method. One (1) sample was repeatedly reactive when tested on the EVOLIS™ but nonreactive when tested manually.

B. Sensitivity

Reactivity in Known HIV-1 Antibody Positive Samples

One thousand three hundred (1300) known HIV-1 antibody positive samples (501 sera and 799 plasma) were tested with the GS HIV Combo Ag/Ab EIA and an FDA licensed HIV-1/HIV-2 EIA. These samples included 1000 retrospective HIV-1 antibody positive samples from the U.S., 200 retrospective HIV-1 antibody positive samples (International) from geographic locations outside the U.S., and 100 samples with CDC AIDS stage classification. Reactivity with the GS HIV Combo Ag/Ab EIA in the 1300 known antibody positive samples is presented in Table 7. Results are also compared to results of testing the same samples with a licensed HIV-1/HIV-2 EIA.

Table 7
Reactivity in Known HIV-1 Antibody Positive Samples

Known HIV Antibody Status	N	GS HIV Combo Ag/Ab EIA Repeatedly Reactive	Licensed HIV-1/HIV-2 EIA Repeatedly Reactive
HIV-1 Antibody Positive (U.S.)	1000	1000	1000
HIV-1 Antibody Positive (International)	200	200	198*
AIDS with CDC Stage Classification	100	100	100
Total	**1300**	**1300**	**1298***

* Two (2) samples were initially reactive but had insufficient volume for repeat testing.

As shown in Table 7, the sensitivity of the GS HIV Combo Ag/Ab EIA in the HIV-1 known positive population was 100% (1300/1300) with a 95% confidence interval of 99.70% - 100%.

Of the 1300 samples that were tested manually with the GS HIV Combo Ag/Ab EIA, 349 samples were tested with the GS HIV Combo Ag/Ab EIA on the EVOLIS™ Automated Microplate System. The results by EVOLIS™ testing were equivalent in 100% (349/349) of the samples from known HIV-1 positive samples when compared to the manual method.

Reactivity in Known HIV-2 Antibody Positive Samples

Two hundred (200) known HIV-2 antibody positive samples obtained from individuals from different geographic locations were tested with the GS HIV Combo Ag/Ab EIA and an FDA licensed HIV-1/HIV-2 EIA. Results are presented in Table 8.

Table 8
Reactivity in Known HIV- 2 Antibody Positive Samples

Known HIV Antibody Status	N	GS HIV Combo Ag/Ab EIA Repeatedly Reactive	Licensed HIV-1/HIV-2 EIA Repeatedly Reactive
HIV-2 Antibody Positive	200	200	200

As shown in Table 8, the sensitivity of the GS HIV Combo Ag/Ab EIA with HIV-2 antibody positive samples was 100% (200/200) with a 95% confidence interval of 98.11% - 100%.

Of the 200 known HIV-2 antibody positive samples that were tested manually with the GS HIV Combo Ag/Ab EIA, 100 were tested with the GS HIV Combo Ag/Ab EIA on the EVOLIS™ Automated Microplate System. The results by EVOLIS™ testing were equivalent in 100% (100/100) of the samples from known HIV-2 positive samples when compared to the manual method.

HIV-1 Group O Antibody Positive Samples

Sixty-three (63) HIV-1 Group O samples were tested with the GS HIV Combo Ag/Ab EIA. Results are summarized in Table 9.

Table 9
HIV-1 Group O Positive Samples

	Country of Origin	N	GS HIV Combo Ag/Ab EIA Repeatedly Reactive
HIV-1 Group O	Cameroon	61	61
	United States	1	1
	France	1	1
	Total	**63**	**63 (100%)**

Of the 63 HIV-1 Group O samples tested with the GS HIV Combo Ag/Ab EIA, 100% (63/63) were repeatedly reactive.

AIDS with CDC Stage Classification

A total of 100 CDC AIDS stage classification samples were tested with the GS HIV Combo Ag/Ab EIA and a licensed HIV-1/HIV-2 EIA. Results are summarized in Table 10.

Table 10
AIDS with CDC Stage Classification

		Repeatedly Reactive	
CDC Stage	**N**	**GS HIV Combo Ag/Ab EIA**	**Licensed HIV-1/HIV-2 EIA**
CDC AIDS Stage A3	11	11 (100%)	11 (100%)
CDC AIDS Stage B3	9	9 (100%)	9 (100%)
CDC AIDS Stage C1	11	11 (100%)	11 (100%)
CDC AIDS Stage C2	20	20 (100%)	20 (100%)
CDC AIDS Stage C3	49	49 (100%)	49 (100%)
Total	**100**	**100** (100%)	**100** (100%)

As shown in Table 10, the sensitivity of the GS HIV Combo Ag/Ab EIA in AIDS patients with CDC Stage Classification was 100% (100/100) with a 95% confidence interval of 96.29% - 100%.

Of the 100 samples with known CDC AIDS stage classifications that were tested manually with the GS HIV Combo Ag/Ab EIA, 50 were tested with the GS HIV Combo Ag/Ab EIA on the EVOLIS™ Automated Microplate System. The results by EVOLIS™ testing were equivalent in 100% (50/50) of the samples from known AIDs CDC stage classification when compared to the manual method.

Individuals with Acute HIV Infection and Follow-Up Sample Testing

Twenty-one (21) frozen serially drawn serum samples from 9 individuals with acute HIV infection were tested in duplicate with the GS HIV Combo Ag/Ab EIA. The mean signal-to-cutoff ratio (S/CO) is presented in Table 11. The GS HIV Combo Ag/Ab EIA results are compared to historical data. The historical data provided include HIV-1 RNA copies, and results from a licensed HIV-1/HIV-2 EIA, a licensed HIV-1 EIA, and a licensed HIV-1 Western blot.

Table 11
Reactivity with Acute HIV Infection
N = 9 Patients, 21 Samples

Acute HIV Patient	Days from 1st bleed	HIV-1 RNA copies/ mL	GS HIV Combo Ag/Ab EIA		Historical Results				
					HIV-1/HIV-2 EIA		HIV-1 EIA		HIV-1 Western blot
			S/CO	Result	S/CO	Result	S/CO	Result	
1	0	>500,000	**15.09**	**RR**	0.38	NR	0.12	NR	Neg
	56	NA	**15.09**	**RR**	**10.6**	**IR**	**6.52**	**IR**	**Pos**
2	0	183,850	**2.97**	**RR**	0.14	NR	0.45	NR	Neg
	16	10,479	**15.09**	**RR**	**10.4**	**IR**	**6.42**	**IR**	**Pos**
	42	NA	**15.09**	**RR**	**10.3**	**IR**	**6.08**	**IR**	**Pos**
3	0	>500,000	**15.09**	**RR**	**8.88***	**RR***	NA	NA	Neg
	141	NA	**15.09**	**RR**	**10.50**	**IR**	**6.42**	**IR**	**Pos**
4	0	>500,000	**15.09**	**RR**	0.02	NR	0.05	NR	Neg
	19	NA	**15.09**	**RR**	**10.20**	**IR**	**2.98**	**IR**	**Pos**
5	0	>500,000	**14.90**	**RR**	**10.99***	**RR***	**1.29***	**RR***	Neg
	21	NA	**15.09**	**RR**	**10.90**	**IR**	**3.70**	**IR**	**Ind**
	64	NA	**15.09**	**RR**	**10.60**	**IR**	**8.59**	**IR**	**Pos**
6	0	795,520	**12.49**	**RR**	0.16	NR	0.05	NR	Neg
	25	NA	**15.09**	**RR**	**10.80**	**IR**	**5.42**	**IR**	**Pos**
	32	NA	**15.09**	**RR**	**10.40**	**IR**	**5.95**	**IR**	**Pos**
7	0	72,000	**2.07**	**RR**	0.08	NR	0.28	NR	Neg
	34	NA	**15.09**	**RR**	**9.87**	**IR**	**6.98**	**IR**	**Pos**
8	0	460,790	**7.19**	**RR**	**1.80***	**RR***	0.15*	NR*	Neg
	15	NA	**15.09**	**RR**	**10.50**	**IR**	**5.01**	**IR**	**Pos**
	29	NA	**15.09**	**RR**	**10.90**	**IR**	**7.57**	**IR**	**Pos**
9	0	20,420	0.31	NR	0.09	NR	0.09	NR	Neg
Total			**20/21 (95.24%)**		**15/21 (71.43%)**		**13/20 (65.00%)**		**11/21 (52.38%)**

NA = Not Available RR = Repeatedly Reactive IR = Initially Reactive NR = Nonreactive Neg = Negative
Pos = Positive Ind = Indeterminate
* These values are the mean of three results.

As shown in Table 11, the GS HIV Combo Ag/Ab EIA was repeatedly reactive with 95.24% (20/21) of the acute HIV-1 infection specimens tested. One sample that appeared to be a very early infection (20,420 RNA copies) was nonreactive with GS HIV Combo Ag/Ab EIA. In comparison for these 21 acute samples, the licensed HIV-1/HIV-2 EIA was reactive with 71.43% (15/21), the licensed HIV-1 EIA was reactive with 65.00% (13/20) and the licensed HIV-1 Western blot was reactive with 52.38% (11/21).

Table 12 is a summary of the GS HIV Combo Ag/Ab EIA reactivity with the nine acute HIV-1 infected patients studied in Table 11 in comparison to three licensed assays (HIV-1/HIV-2 EIA, HIV-1 EIA, and HIV-1 Western blot).

Table 12
Acute Infection - Assay Comparison Summary

	# of Specimens for which GS HIV Combo Ag/Ab EIA is:		
Comparator Assay	**More Sensitive**	**Equivalent**	**Less Sensitive**
vs. Licensed HIV-1/HIV-2 EIA	5/9 (55.56%)	4/9 (44.44%)	0/9 (0.00%)
vs. Licensed HIV-1 EIA	6/9 (66.67%)	3/9 (33.33%)	0/9 (0.00%)
vs. Licensed HIV-1 Western blot	8/9 (88.89%)	1/9 (11.11%)	0/9 (0.00%)

Overall, the GS HIV Combo Ag/Ab EIA was more sensitive in reactivity with the acute HIV infection specimens than all three licensed assays used in the comparison. The GS HIV Combo Ag/Ab EIA was more sensitive than the HIV-1/HIV-2 EIA for 55.56% (5/9) of the samples, more sensitive than the HIV-1 EIA for 66.67% (6/9) of the samples and more sensitive than the HIV-1 Western blot for 88.89% (8/9) of the samples.

HIV-1 NAT-yield Samples

Thirty (30) HIV-1 NAT-yield plasma samples from a U.S. blood bank (mainly clade B) were evaluated with the GS HIV Combo Ag/Ab EIA and a licensed HIV-1/HIV-2 EIA. In this testing, 33.33% (10/30) of the HIV-1 NAT-yield plasma samples were reactive with the GS HIV Combo Ag/Ab EIA by the manual method and 36.67% (11/30) were reactive using the EVOLIS™ Automated Microplate System. One sample near the cutoff (mean S/CO 0.97) by the manual method was reactive (mean S/CO 1.14) using EVOLIS™. In comparison, 10.00% (3/30) were reactive with the licensed HIV-1/HIV-2 EIA, a 3rd generation HIV antibody test. All samples that were reactive on the licensed HIV-1/HIV-2 EIA were also reactive on the GS HIV Combo Ag/Ab EIA.

An additional 71 HIV-1 NAT-yield plasma samples from blood banks outside the U.S. were tested with the GS HIV Combo Ag/Ab EIA and a licensed HIV-1/HIV-2 EIA. These HIV-1 NAT-yield plasma samples included subtypes A1 and C, and they were from South Africa, Germany, Poland and France. In this study, 45.07% (32/71) of the HIV-1 NAT-yield plasma samples were reactive with the GS HIV Combo Ag/Ab EIA in comparison to 8.45% (6/71) that were reactive with the licensed HIV-1/HIV-2 EIA. All samples that were reactive on the licensed HIV-1/HIV-2 EIA were also reactive on the GS HIV Combo Ag/Ab EIA.

p-NAAT (Pooled Nucleic Acid Amplification Test) Screening Samples

The GS HIV Combo Ag/Ab EIA was evaluated in the testing of 50 retrospective p-NAAT screening samples (using a research NAT). Forty-four (44) were known positive for HIV and 6 were known negative. In this testing, 86.36% (38/44) HIV-1 p-NAAT positive screening samples were reactive with the GS HIV Combo Ag/Ab EIA in comparison to 59.09% (26/44) that were reactive with a 3rd generation HIV-1/HIV-2 antibody test. All samples that were reactive on the licensed HIV-1/HIV-2 EIA were also reactive on the GS HIV Combo Ag/Ab EIA.

Individuals at High Risk for HIV Infection - STD Clinic

A total of 1000 samples obtained from individuals at high risk for HIV infection were tested with the GS HIV Combo Ag/Ab EIA and a licensed HIV-1/HIV-2 EIA. The plasma samples were from individuals belonging to groups recognized to be at a higher risk for HIV infection due to the following factors: lifestyle, behavior, or known exposure event. All samples repeatedly reactive on either test were tested by a licensed HIV-1 Western blot and an FDA approved HIV-1/HIV-2 test that differentiates HIV-1 from HIV-2. Results are presented in Table 13.

Table 13
Reactivity in Individuals at High Risk for HIV Infection

GS HIV Combo Ag/Ab EIA	Licensed HIV-1/HIV-2 EIA		
	Repeat Reactive	**Nonreactive**	**Total**
Repeat Reactive	41*	0	41
Nonreactive	0	959	959
Total	**41**	**959**	**1000**

* 40/41 samples that were repeat reactive with both the GS HIV Combo Ag/Ab EIA and the licensed HIV-1/HIV-2 EIA were HIV-1 Western Blot positive; one sample was HIV-1 Western blot negative and also negative on an approved HIV-1/HIV-2 differentiation test and HIV-1 RNA negative.

As shown in Table 13, 41/1000 (4.10%) individuals at high risk for infection were repeatedly reactive with the GS HIV Combo Ag/Ab EIA. Of the 41 repeatedly reactive samples, 40 samples were positive by HIV-1 Western blot and by the HIV-1/HIV-2 differentiation test. All samples that were repeat reactive by the licensed HIV-1/HIV-2 EIA and HIV-1 Western blot positive were repeat reactive on the GS HIV Combo Ag/Ab EIA. The GS HIV Combo Ag/Ab EIA was repeatedly reactive in 100% (40/40) of the HIV positive samples in this high risk population with a 95% confidence interval of 91.22% - 100%.

Of the 1000 samples from high risk individuals that were tested manually with the GS HIV Combo Ag/Ab EIA, 504 samples (485 nonreactive and 19 repeat reactive) were also tested with the GS HIV Combo Ag/Ab EIA on the EVOLIS™ Automated Microplate System. The results by EVOLIS™ testing were equivalent in 100% (504/504) of the samples from high risk individuals as compared to the manual method.

Individuals from an HIV-2 Endemic Region

A total of 500 samples from an HIV-2 endemic region (Sierra Leone and Guinea Bissau) were tested with the GS HIV Combo Ag/Ab EIA and a licensed HIV-1/HIV-2 EIA. Repeatedly reactive samples were tested by a licensed HIV-1 Western blot and an HIV-1/HIV-2 differentiation test kit. Results are presented in Table 14 and Table 15.

Table 14
Reactivity in Individuals from HIV-2 Endemic Region

High Risk Population	N	GS HIV Combo Ag/Ab EIA Repeat Reactive	Licensed HIV-1/HIV-2 EIA Repeat Reactive	Repeatedly Reactive Specimens						
				HIV-1 Western Blot			HIV Differentiation Test			
							Reactive			
				Pos	Ind	Neg	HIV-1	HIV-2	Not Differentiated	NR
HIV-2 Endemic Region	500	150 (30.00%)	144 (28.80%)	118[a] (23.60%)	24[b] (4.80%)	8[c] (1.60%)	102 (20.40%)	18[d] (3.60%)	5[e] (1.00%)	25 (5.00%)

[a] Of these 118 samples positive for HIV-1 on an FDA-licensed HIV-1 Western blot, results with an FDA-approved HIV differentiation test were as follows: 102 were reactive for antibodies to HIV-1, 10 were reactive for antibodies to HIV-2, 5 were undifferentiated, and 1 was nonreactive.
[b] Of these 24 samples Indeterminate for HIV-1 on the FDA-licensed HIV-1 Western blot, 8 were reactive for antibodies to HIV-2 and 16 were nonreactive with the FDA-approved HIV differentiation test.
[c] All 8 HIV-1 Western blot negative samples were nonreactive with the FDA-approved HIV differentiation test.
[d] Many of the 18 samples that were HIV-2 reactive by the FDA-approved HIV differentiation test exhibited atypical patterns on the FDA-licensed HIV-1 Western blot.
[e] All 5 undifferentiated samples were positive on the FDA-licensed HIV-1 Western blot.

Table 15
Reactivity in Individuals from HIV-2 Endemic Region - Assay Comparison Summary

GS HIV Combo Ag/Ab EIA	Licensed HIV-1/HIV-2 EIA		
	Repeat Reactive	**Nonreactive**	**Total**
Repeat Reactive	139	11[a]	150[b]
Nonreactive	5[c]	345	350
Total	**144**	**356**	**500**

[a] In HIV-1 Western blot testing, 0/11 samples were positive, 8/11 were indeterminate, and 3/11 were negative. On the approved HIV differentiation test, all were nonreactive.
[b] In HIV-1 Western blot testing, 118/150 samples were positive, 24/150 were indeterminate, and 8/150 were negative. On the approved HIV differentiation test, 102 were HIV-1 reactive, 18 were HIV-2 reactive, and 5 were undifferentiated.
[c] In HIV-1 Western blot testing, 0/5 samples were positive, 1/5 were indeterminate, and 4/5 were negative. On the approved HIV differentiation test, all were nonreactive.

As shown in Tables 14 and 15, 150/500 (30.00%) samples in this HIV-2 endemic population were repeatedly reactive with the GS HIV Combo Ag/Ab EIA. Of the 150 GS HIV Combo Ag/Ab EIA repeatedly reactive, 126 samples were also positive by the licensed HIV-1 Western blot or the approved HIV differentiation test (102 were HIV-1 positive by both HIV-1 Western blot and the HIV differentiation test, 6 samples were HIV-1 reactive by the HIV-1 Western blot only, and 18 samples were HIV-2 reactive with the HIV differentiation test.

Of the 500 samples from an HIV-2 endemic region tested manually with the GS HIV Combo Ag/Ab EIA, 100 samples were tested with the GS HIV Combo Ag/Ab EIA on the EVOLIS™ Automated Microplate System. The results by EVOLIS™ testing were equivalent in 99.00% (99/100) of the HIV-2 endemic region samples compared to the manual method. One (1) sample was repeatedly reactive when tested on the EVOLIS™ but nonreactive (near the cutoff) when tested manually. This sample was HIV-1 Western blot indeterminate, negative with the HIV differentiation test, and HIV-1 Ag nonreactive.

C. Pediatric Populations (2-20 years)

Specificity in Low and High Risk Pediatric Populations

The reactivity rate of the GS HIV Combo Ag/Ab EIA was determined using 100 plasma samples from healthy pediatric subjects (age 2 - 21 years), 87 samples from pregnant females (age 15 - 21 years), 125 blood donors (age 16 - 21 years), 34 samples from a low risk population (age 3 – 21 years), and 57 samples from a high risk U.S. population that were collected at an STD clinic (age 18 - 21 years), for a total of 403 samples from a pediatric population at low and high risk for HIV infection. Samples were tested with the GS HIV Combo Ag/Ab EIA and a licensed HIV-1/HIV-2 EIA. Results are presented in Table 16.

Table 16
Low and High Risk Pediatric Population

Age Range In Years	Gender	N	GS HIV Combo Ag/Ab EIA		FDA Licensed HIV-1/HIV-2 EIA		Confirmed Positive Samples
			Non-Reactive	Repeatedly Reactive	Non-Reactive	Repeatedly Reactive	
2 - 5	**Female**	8	8	0	8	0	0
	Male	8	8	0	8	0	0
6 - 10	**Female**	7	7	0	7	0	0
	Male	11	11	0	11	0	0
11 - 15	**Female**	24	24	0	24	0	0
	Male	21	21	0	21	0	0
16 - 21	**Female**	210	209	1	210	0	0
	Male	114	111	3	111	3	3
		403	**399 (99.01%)**	**4 (0.99%)**	**400 (99.26%)**	**3 (0.74%)**	**3 (0.74%)**

The specificity of the GS HIV Combo Ag/Ab EIA in the low and high risk pediatric population was 99.75% (399/400) with a 95% confidence interval of 98.60% - 99.96%.

Reactivity of the GS HIV Combo Ag/Ab EIA in Known Positive Pediatric Subjects

The reactivity rate of the GS HIV Combo Ag/Ab EIA was determined for known positive specimens from pediatric subjects (ranging in age from 2 – 21 years). A total of 41 (32 serum and 9 plasma) frozen retrospective HIV-1 antibody positive pediatric samples were tested with the GS HIV Combo Ag/Ab EIA and a licensed HIV-1/HIV-2 EIA. Repeatedly reactive samples on either of these assays were also tested with a licensed HIV-1 Western blot. Results are presented in Table 17.

Table 17
HIV-1 Antibody Positive Pediatric Samples

Age Range In Years	Gender	N	GS HIV Combo Ag/Ab EIA		Licensed HIV-1/HIV-2 EIA		HIV-1 Western blot Positive
			Non-Reactive (%)	Repeatedly Reactive (%)	Non-Reactive (%)	Repeatedly Reactive (%)	
2 - 5	**Male**	2	0	2	0	2	2
6 - 10	**Female**	3	0	3	0	3	3
	Male	6	0	6	0	6	6
11 - 15	**Female**	7	0	7	0	7	7
	Male	3	0	3	0	3	3
16 - 21	**Female**	7	0	7	0	7	7
	Male	13	0	13	0	13	13
Total		**41**	**0 (0.00%)**	**41 (100.00%)**	**0 (0.00%)**	**41 (100.00%)**	**41(100.00%)**

As can be seen in Table 16, 100% (41/41) of the known HIV-1 antibody positive pediatric samples were repeatedly reactive with the GS HIV Combo Ag/Ab EIA. All of the 41 repeatedly reactive samples were positive for antibodies to HIV-1 by Western blot.

IX. Conclusions Drawn from the Studies

The GS HIV Combo Ag/Ab EIA detects circulating antibodies to HIV-1 (Groups M and O) and HIV-2, and it also detects HIV p24 antigen. The performance of the GS HIV Combo Ag/Ab EIA was evaluated in a multi-centered clinical study conducted in the U.S., and results were compared to HIV antibody assays that are currently FDA-licensed or approved.

- The specificity of the GS HIV Combo Ag/Ab EIA in the low risk populations was 99.87% (6987/6996).
- The sensitivity of the GS HIV Combo Ag/Ab EIA in the HIV-1 known positive populations was 100% (1300/1300), and the sensitivity in individuals at high risk for HIV-1 infection was 100% (40/40). The sensitivity of the GS HIV Combo Ag/Ab EIA with HIV-2 antibody positive samples was 100% (200/200). In an HIV-2 endemic population, 100% (126/126) of the HIV positive samples were detected.
- The antigen sensitivity of the GS HIV Combo Ag/Ab EIA with the antigen standard derived from the AFSSAPS was 14.78 pg/mL (range of 13.22 - 15.89 pg/mL). The antigen sensitivity of the GS HIV Combo Ag/Ab EIA with the WHO HIV-1 p24 antigen international standard was 0.65 IU/mL (range of 0.40 – 1.05 IU/mL).
- Of the 53 HIV-1 culture supernatant samples of subtypes A, B, C, D, AE, F, G, H, J, N and O tested, 100% (53/53) were reactive.
- In the testing of 30 commercial seroconversion panels, the GS HIV Combo Ag/Ab EIA detected reactive bleeds in 96.67% (29/30) of the seroconversion panels compared to 83.33% (25/30) detected by the FDA licensed HIV-1/HIV-2 EIA and 23.33% (7/30) detected by the HIV-1 Western blot.
- Of the 63 HIV-1 Group O samples tested with the GS HIV Combo Ag/Ab EIA, 100% (63/63) were reactive.

- An HIV-1 Group M subtype panel was repeatedly reactive on 100% (15/15) of the members, an HIV-1 Low Titer Panel was reactive on 100% (14/14) of the members, and an HIV-1 Incidence / Prevalence Panel was 100% reactive (15/15 members).
- The sensitivity of the GS HIV Combo Ag/Ab EIA in AIDS patients with CDC Stage Classification was 100% (100/100).
- The GS HIV Combo Ag/Ab EIA was reactive with 95.24% (20/21) of the acute HIV-1 infection specimens tested. One sample from one patient that appeared to be a very early infection (20,420 RNA copies) was nonreactive with GS HIV Combo Ag/Ab EIA.
- Of 30 HIV-1 NAT-yield plasma samples from a U.S. blood bank (mainly clade B) evaluated, 33.33% (10/30) were reactive. Of 71 HIV-1 NAT-yield plasma samples from blood banks outside the U.S. (including subtypes A1 and C), 45.07% (32/71) were reactive.
- In the testing of retrospective p-NAAT screening samples, 86.36% (38/44) of the known positive samples were reactive with the GS HIV Combo Ag/Ab EIA.
- The specificity of the GS HIV Combo Ag/Ab EIA in a low and high risk pediatric population was 99.75% (399/400), and in pediatric known HIV-1 antibody positive samples, 100% (41/41) were repeatedly reactive.

The performance characteristics of the assay are not affected by potential cross-reacting substances that may be present in clinical samples, or by interfering substances (hemoglobin, lipemia, bilirubin, or protein levels).

Blood collection tube studies support the use of human serum in standard glass or plastic tubes and plasma (EDTA [K2], sodium and lithium heparin, and sodium citrate) as well as serum collected into serum separator tubes (with and without activator) and plasma with lithium heparin collected into plasma separator tubes in testing with the GS HIV Combo Ag/Ab EIA.

Stability studies demonstrate that, when stored as indicated (2-8°C), the GS HIV Combo Ag/Ab EIA is stable for the intended shelf-life of the kits.

Risk/Benefit Analysis

As a diagnostic test, the GS HIV Combo Ag/Ab EIA involves removal of blood from an individual for testing purposes. This test presents no more of a safety hazard to an individual than is presented to an individual who is having their blood drawn for any other diagnostic evaluation. The benefit to HIV-1 or HIV-2 infected individuals tested by this assay outweighs any potential adverse event or risk to the patient or user due to assay malfunction or operator error.

The potential risks encountered with *in vitro* diagnostic tests are not unusual in the clinical laboratory setting. Appropriate warnings for these risks are contained in the labeling and package inserts for these devices. Standard good laboratory practices are considered sufficient to mitigate the risks to the end user.

Safety

There were no adverse reactions observed in any of the clinical or pre-clinical studies that were conducted with the GS HIV Combo Ag/Ab EIA. When testing is performed as specified in the assay product instructions and results are used in combination with clinical findings, the test can be considered safe and effective.

Reactive specimens must be investigated by additional, more specific, or supplemental tests. Both confirmation of the test result on a freshly drawn sample and counseling should be considered an important part of testing for HIV antigen and antibody to HIV-1 and HIV-2. 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. 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.

Effectiveness

The effectiveness of the GS HIV Combo Ag/Ab EIA has been shown in the clinical and pre-clinical studies that have been performed with serum and plasma samples. Based on these results, the assay has been shown to be an effective tool in evaluating patients with signs or symptoms of AIDS, and in establishing prior infection with HIV-1 or HIV-2

X. Panel Recommendations

Not Applicable – This product was not submitted for review by the Blood Products Advisory Committee.

XI. FDA/CBER Decision

XII. Approval Specifications

None