Summary of Safety and Effectiveness
Calypte Biomedical Corporation's
Cambridge Biotech HIV-1 Urine Western Blot

I. General Information

Device Generic Name:	In vitro qualitative assay for the detection and identification of antibodies to Human Immunodeficiency Virus (Type 1 (HIV-1) in Urine
Device Trade Name:	Calypte Biomedical Corporation's Cambridge Biotech HIV-1 Urine Western Blot Kit
Applicant's Name and Address:	Corporate Offices Calypte Biomedical Corporation 1265 Harbor Bay Parkway Alameda, CA 94502 Kit Manufacturing Location Calypte Biomedical Corporation 1500 E. Gude Drive Rockville, MD 20850 Controls Manufacturing Location Calypte Biomedical Corporation 1265 Harbor Bay Parkway Alameda, CA 94502
PMA Number:	
Date of Panel Recommendation:	None
Date of Good Manufacturing Inspection:	None

II. Indications for Use

The Cambridge Biotech HIV-1 Urine Western Blot Kit is an *in vitro* qualitative assay for the detection and identification of antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) in human urine. This more specific assay is used as a supplemental test with urine specimens that tested repeatedly reactive using a screening procedure (the Calypte HIV-1 Urine EIA).

III. Background/Device Description

Summary and Explanation of the Test

The Enzyme-Linked Immunosorbent Blot Technique ("Western Blot") has been used to detect antibodies to Human Immunodeficiency Virus Type 1 (HIV-1), which has been recognized as the etiological agent of the Acquired Immunodeficiency Syndrome (AIDS). The combination of electrophoretic separation of complex mixtures of antigens with the highly sensitive immunoblotting technique has been useful in characterizing the antigenic profile of HIV-1 and describing the immune response to this virus in exposed or infected persons.

The Cambridge Biotech HIV-1 Western Blot Kit, when used as directed in this insert, will detect antibodies to HIV-1 when present in human serum, plasma, or urine. The position of bands on the nitrocellulose strips allows this antibody reactivity to be associated with specific viral antigens.

Persons demonstrating antibodies to HIV-1 should be referred for medical evaluation, which may include testing by other techniques. A clinical diagnosis of AIDS can be made only if a person meets the case definition of AIDS established by the Centers for Disease Control.

Like most other chronic diseases, AIDS is a complicated multifactorial, multistep process, with HIV-1 infection being a principal component. Accurate diagnosis of HIV-1 infection is important in determining an individual's risk for developing AIDS. Accuracy is complicated by false-positive and false-negative (EIA) results. It would appear that in some limited infections, a compartmentalized response occurs in which expression of HIV-1 or its respective immune response is limited to a restricted number of organs and tissues.

Principles of the Procedure

The Cambridge Biotech HIV-1 Western Blot Kit is manufactured by Calypte Biomedical from HIV-1 propagated in an H9/HTLV-III_B T-Lymphocyte cell line. The partially purified virus is inactivated by treatment with psoralen and ultraviolet light, and detergent disruption. Specific HIV-1 proteins are fractionated according to molecular weight by electrophoresis on a polyacrylamide slab gel in the presence of sodium dodecylsulfate (SDS). The separated HIV-1 proteins are electrotransferred from the gel to a nitrocellulose membrane which is then washed, blocked (to minimize nonspecific immunoglobulin binding), and packaged. Individual nitrocellulose strips are incubated with specimens and controls. During incubation, if HIV-1 antibodies are present in the specimen, they will bind to the viral antigens present on the nitrocellulose strips. The strips are washed again to remove unbound material. Visualization of the human immunoglobulins specifically bound to HIV-1 proteins is accomplished *in situ* using a series of reactions with goat anti-human IgG conjugated with biotin, avidin conjugated with horseradish peroxidase (HRP), and the HRP substrate, 4-chloro-1-naphthol. If antibodies to any of the major HIV-1 antigens are present in the specimen in sufficient concentration, bands corresponding to the position of one or more of the following HIV-1 proteins (p) or glycoproteins (gp) will be seen on the nitrocellulose strip: p17, p24, p31, gp41, p51, p55, p66, gp120, gp160 (the number refers to the apparent molecular weight in kilodaltons).

Reagents

1 NITROCELLULOSE STRIPS - Each NITROCELLULOSE STRIP contains separated, bound antigenic proteins from partially purified, inactivated HIV-1, in sufficient quantity to detect human antibodies. Bovine protein is present as a blocking agent. Strips are consecutively numbered (1 through 27).

2 NEGATIVE URINE CONTROL - Inactivated human urine negative for antibodies to HIV-1 antigens. A serum sample from the source was non-reactive for hepatitis B surface antigen and hepatitis C antibodies. Contains 0.1% sodium azide as a preservative.

3 LOW POSITIVE URINE CONTROL - Inactivated human urine positive for antibodies to HIV-1 antigens. A serum sample from the source was non-reactive for hepatitis B surface antigen and hepatitis C antibodies. Contains 0.1% sodium azide as a preservative.

4 HIGH POSITIVE URINE CONTROL - Inactivated human urine containing a high titer of antibodies to HIV-1 antigens. A serum sample from the source was non-reactive for hepatitis B surface antigen and hepatitis C antibodies. Contains 0.1% sodium azide as a preservative.

5 WASH BUFFER - Supplied as a 20x concentrate. When diluted, this reagent contains 0.02 M tris, 0.1 M NaCl, 0.3% Tween 20, and 0.005% thimerosal as a preservative, at pH 7.4.

6 BLOTTING BUFFER - Supplied as a 10x concentrate. When diluted, this reagent contains 0.02 M tris, 0.1 M NaCl, heat-inactivated normal goat serum, and 0.01% thimerosal as a preservative, at pH 7.4.

7 CONJUGATE 1 - Biotinylated Goat Anti-human IgG (heavy and light chain) antibodies. Contains 0.002% thimerosal as a preservative.

8 CONJUGATE 2 - Avidin conjugated horseradish peroxidase. Contains 0.01% thimerosal as a preservative.

9 SUBSTRATE A - 7.8 mM solution of 4-chloro-1-naphthol in an alcohol solution.

10 SUBSTRATE B - Aqueous hydrogen peroxide solution (0.02%) in citrate buffer.

11 BLOTTING POWDER - nonfat dry milk.

IV. Alternative Practices and Procedures

There are no other products currently in commercial distribution in the United States designed to detect antibodies to HIV-1 in human urine other than a screening test, the Calypte HIV-1 Urine EIA (Calypte Biomedical Corporation). Several products are available to detect antibodies to HIV in human serum, plasma, dried blood spots and oral fluid.

V. Marketing History

The Cambridge Biotech HIV-1 Western Blot was originally licensed by FDA on 4/30/1987. It was manufactured at that time by Biotech Research Laboratories and distributed by E.I. DuPont de Nemours Company. The product, facilities and license were subsequently transferred to Cambridge Biotech Corporation under US license number 1063. Approval for the urine testing application of the Cambridge Biotech HIV-1 Western blot product was obtained by Cambridge Biotech Corporation on 5/28/98 as a license supplement (95-1588). Calypte Biomedical Corporation acquired certain assets of Cambridge Biotech Corporation (then a part of bioMerieux Vitek, Inc.) on 12/18/1998. These assets included the Cambridge Biotech HIV-1 Western Blot product which was transferred to Calypte Biomedical Corporation's Biologics License 1207. The urine application for the Cambridge Biotech HIV-1 Western Blot has been in commercial distribution by Calypte in the United States since that date. The product has had limited distribution outside of the United States.

VI. Summary of Studies

Nonclinical Laboratory Studies

Expiration dating periods for each kit component have been established as shown below.

Nitrocellulose strips – 9 months
Wash Buffer (20X) – 12 months
Blotting Buffer (10X) – 12 months
Blotting Powder – 12 months
Conjugate #1 – 9 months
Conjugate #2 – 9 months
Substrate A – 9 months
Substrate B – 9 months
Negative Urine Control – 12 months
Low Positive Urine Control – 12 months
High Positive Urine Control – 12 months

Clinical Studies

Three studies were conducted to evaluate the performance of the Cambridge Biotech HIV-1 Urine Western Blot Kit. The performance was evaluated by comparing the results of urine specimens to the results of paired serum specimens tested with a licensed HIV-1 Western Blot Kit.

One study (Study 1) evaluated 696 archived urine specimens. The specimens were from low risk (N=200), high risk (N=37) and HIV-1 positive (N=377) populations. The HIV-1 positive populations included patients symptomatic (N=55) and asymptomatic (N=87) for HIV-1

infection, AIDS patients (N=115) and HIV-1 positive subjects from foreign sites (N=120) whose clinical status was unknown. Other specimens (N=82) were obtained and evaluated from subjects with medical conditions unrelated to HIV-1 infection that might result in antibodies cross-reactive with HIV-1 proteins.

The Cambridge Biotech HIV-1 Urine Western Blot results compared to serum Western Blot results are presented in Table A. Two additional studies (Study 2, Study 3) evaluated 1,240 prospectively collected urine specimens. Study 2 evaluated specimens from subjects whose HIV-1 clinical status was unclassified (N=197), subjects who were HIV-1 negative but at high risk of HIV-1 infection (N=51) and subjects with non-HIV related medical conditions (N=1). Study 3 evaluated low risk (N=315), high risk (N=303) and HIV-1 positive (N=175) populations, including AIDS patients. The HIV-1 positive populations included patients symptomatic (N=38) and asymptomatic (N=36) for HIV-1 infection and AIDS patients (N=101). Other specimens (N=198) were also obtained from subjects with unrelated medical conditions that might result in assay interference.

In the three studies combined, 1,936 paired urine and serum specimens collected from multiple geographical locations within the United States and from foreign sites were evaluated at four testing laboratories throughout the United States. The status of the subject was based upon the paired serum result or documented clinical status of the subject.

Two additional special studies were conducted using specimens from Study 3 to assess the performance of the Cambridge Biotech HIV-1 Western Blot kit. One evaluation involved Western blot testing of urine specimens paired to serum EIA non-reactive Western blot indeterminate specimens (N=109). The second evaluation involved urine Western blot testing of urine EIA repeatedly reactive specimens paired to serum EIA non-reactive specimens from uninfected individuals (N=114).

Sensitivity In HIV-1 Seropositive Individuals

The sensitivity of the Cambridge Biotech HIV-1 Western Blot Kit using urine was evaluated by comparing the urine results to the results obtained from testing paired serum specimens collected from individuals who were HIV-1 seropositive and from individuals clinically diagnosed as AIDS patients. The results of this study is shown in Table A.

In the combined studies, the Cambridge Biotech HIV-1 Western Blot Kit using urine obtained from the 215 patients clinically diagnosed with AIDS identified 213 of 215 (99.1%) patients as Western Blot positive. There were two (2) false negative urine Western Blot results in the AIDS population. The Cambridge Biotech HIV-1 Western Blot Kit using urine obtained from HIV-1 positive symptomatic, asymptomatic and unclassified groups correctly identified 533 of 533 (100%) patients as Western Blot positive. In the combined population of AIDS patients and other HIV-1 positive patients tested in this study, the Cambridge Biotech HIV-1 Western Blot Kit using urine correctly identified 746 of 748 (99.7%) patients as positive.

Table A
Urine and Paired Serum Western Blot Results for Confirmed HIV-1 Seropositive Individuals and AIDS Patients (N=748)

Risk Group	N	Serum Western Blot Results[f]			Urine Western Blot Results		
		Pos[i]	Neg	Ind	Pos	Neg	Ind
AIDS[a]	215	215	0	0	213	2	0
Sympt[b]	93	93	0	0	93	0	0
Asympt[c]	123	122	0	1[h]	123	0	0
Un-classified[d, e]	317	296[g]	0	1[h]	317	0	0
Total	748	726	0	2	746	2	0

[a] One hundred and fifteen (115) specimens from Study 1, 100 specimens from Study 3.
[b] Fifty-five (55) specimens from Study 1, 38 from Study 3.
[c] Eighty-seven (87) specimens from Study 1, 36 from Study 3.
[d] One hundred twenty (120) specimens from Study 1, 197 from Study 2.
[e] The clinical status of these HIV-1 positive subjects was unknown.
[f] A licensed serum HIV-1 Western Blot Kit was used when testing serum specimens.
[g] Twenty (20) of the 316 specimens were from Uganda and were not confirmed by Western blot. The specimens were confirmed by a second manufacturer's EIA and by agglutination.
[h] The specimen did not meet the required band intensity criterion for a positive on serum Western blot and therefore was discordant with urine Western blot. However, the patient was known positive by previous clinical diagnosis.
[i] No intensity criterion associated with the LOW POSITIVE URINE CONTROL was used during this analysis.

Sensitivity in High Risk Populations

The sensitivity was also determined in 391 individuals at high risk of HIV-1 infection but of unknown HIV status. Of the 391 high risk subjects tested, 327 were substance abusers and 64 were prostitutes, bisexuals, homosexuals, and other individuals with acknowledged risk factors. The results obtained are provided in Table B.

The results of testing urine specimens from these high risk populations showed that seventeen (17) of seventeen (17) (100%) urine Western blot specimens were correctly identified as positive when compared to the paired serum Western blot results. Of the twenty (20) urine Western blot positives, three (3) urine specimens were paired to serum EIA non-reactive specimens (urine false positives). While the significance of urine positivity in the absence of serum reactivity is not known, the results for these three samples must be classified as false positive in the absence of follow-up testing or clinical information to resolve the infection status of these individuals (see Table B, footnote f).

Sixty-nine (69) specimens were urine EIA repeatedly reactive and urine Western blot negative and were paired to serum EIA non-reactive specimens.

One (1) specimen was urine EIA repeatedly reactive and urine Western blot indeterminate and was paired to a serum EIA non-reactive specimen.

Three hundred (300) urine specimens were EIA non-reactive and Western blot negative and were paired to serum EIA non-reactive specimens. The one urine EIA non-reactive specimen that was Western blot indeterminate was paired to a serum EIA non-reactive specimen.

In this study the sensitivity of the urine Western blot was 100% (17 of 17) for seropositive individuals. In these high risk populations, the specificity of the urine Western blot for EIA repeatedly reactive urine specimens was 94.5% (69 of 73) for seronegative individuals.

Table B
Comparison of Cambridge Biotech HIV-1 Western Blot Results
Using Urine and Paired Serum for High Risk Populations
(N=391)

Risk Group	Serum				Urine			
	EIA		Western Blot[b,d]		EIA		Western Blot	
		N		N		N		N
High	RR	17	Pos	17	RR	90	Pos[e]	20[f]
			Ind	0			Ind	1[a]
			Neg	0			Neg	69
	NR	374	Pos	0	NR	301	Pos	0
			Ind	123			Ind	1[c]
			Neg	230			Neg	300[g]
			NT	20[h]			NT	0
			UNR	1[i]			UNR	0

[a] Non viral bands
[b] A licensed HIV-1 Western Blot Kit was used when testing serum specimens.
[c] p24 only
[d] Serum EIA non-reactive specimens from Study 1 (N=20 drug abusers) were not tested by Western blot.
[e] No intensity criterion associated with the LOW POSITIVE URINE CONTROL was used during this analysis.
[f] Three (3) urine specimens were Western blot positive and paired to serum EIA non-reactive specimens (urine false positives).
[g] Two hundred twenty-nine (229) were correctly classified as negative when compared to the paired serum Western blot results.
[h] NT - Not Tested
[i] UNR - Unreadable

Frequency of Virus Specific Bands in High Risk Populations

The frequency of virus specific bands and interpretation of results for urine specimens tested with the Cambridge Biotech HIV-1 Western Blot Kit from these high risk populations are presented in Table C.

The results show that in these combined high risk populations of 391 specimens, only one of the 301 (0.3%) urine EIA non-reactive specimens demonstrated any viral bands. The band present was p24, resulting in an indeterminate interpretation. Twenty (20) of 90 (22.2%) urine EIA repeatedly reactive specimens were identified as positive on the basis of the presence of the gp160 band. Seventeen (17) of the 20 (85%) urine Western blot positive specimens were paired to serum Western Blot confirmed HIV-1 positive specimens. One (1) urine specimen was paired to a serum Western Blot negative specimen and two (2) were paired to serum Western Blot indeterminate specimens. One (1) of the urine EIA repeatedly reactive specimens was indeterminate due to the presence of non-viral bands on the blot. Sixty-nine (69) urine EIA repeatedly reactive specimens were correctly classified as Western blot negative based on the paired serum results. The serum EIA results were non-reactive.

Table C
Frequency of Virus Specific Bands and Interpretation of Results of
Urine Specimens from a High Risk Population
Tested by the Cambridge Biotech HIV-1 Western Blot Kit
(N=391[a])

Urine EIA	Urine WB		Frequency of Virus Specific Bands[b]								
		N	p17	p24	p31	gp41	p51	p55	p66	gp120	gp160
NR[c]	Pos[g]	0	0	0	0	0	0	0	0	0	0
N=301	Ind	1	0	1	0	0	0	0	0	0	0
RR[d]	Pos[g]	20	1	8	8	16	8	1	16	17	20[f]
N=90	Ind	1[e]	0	0	0	0	0	0	0	0	0

[a] Thirty seven (37) specimens from Study 1, 51 specimens from Study 2, 303 specimens from Study 3.
[b] Band patterns for negative specimens do not appear in this table. By definition, negative specimens show no reactivity.
[c] NR indicates non-reactive in the Calypte Biomedical HIV-1 Urine EIA.
[d] RR indicates repeatedly reactive in the Calypte Biomedical HIV-1 Urine EIA.
[e] Specimen with non-viral bands.
[f] Three (3) of the 20 urine specimens had only a gp160 or gp120 and gp160 band present. All 3 were from Study 1.
[g] No intensity criterion associated with the LOW POSITIVE URINE CONTROL was used during this analysis.

Frequency of Virus Specific Bands in AIDS Patients

Specimens from 215 AIDS patients were tested. Table D presents the frequency of viral specific bands observed and interpretation of results for these AIDS patients.

The results show that 213 of 215 (99.1%) specimens from AIDS patients were positive on the basis of the presence of a gp160 band when tested with the Cambridge Biotech HIV-1 Western Blot Kit.

Table D
Frequency of Virus Specific Bands and Interpretation of Results of Urine Specimens from AIDS Patients
(N=215[a])

Urine EIA	Urine WB		Frequency of Virus Specific Bands[b]								
		N	p17	p24	p31	gp41	p51	p55	p66	gp120	gp160
RR[c]	Pos[e]	213	50	104	130	199	114	44	166	210	213[d]
N=215	Neg	2	0	0	0	0	0	0	0	0	0
	Ind	0	0	0	0	0	0	0	0	0	0

[a] One hundred and fifteen (115) specimens from Study 1, 100 specimens from Study 3.
[b] Band patterns for negative specimens do not appear in this table. By definition, negative specimens show no reactivity.
[c] RR indicates repeatedly reactive in the Calypte Biomedical HIV-1 Urine EIA.
[d] Fifteen (15) of the 213 urine specimens had only a gp160 or gp120 and gp160 band present (11 from Study 1, 4 from Study 3).
[e] No intensity criterion associated with the LOW POSITIVE URINE CONTROL was used during this analysis.

Frequency of Virus Specific Bands in Non-AIDS HIV-1 Positive Populations

The frequency of virus specific bands in non-AIDS HIV-1 populations (N=533) was also determined. The frequency of virus specific bands in HIV-1 symptomatic, asymptomatic and unclassified HIV-1 positive patients (120 foreign specimens from Study 1, 197 HIV-1 positive patients from Study 2) is given in Table E.

All 533 specimens were serum EIA repeatedly reactive and 531 serum Western blot positive, 2 were serum Western blot indeterminate. The results in Table I demonstrate the presence of a gp160 band in 533 of 533 of the HIV-1 positive urine specimens tested with the Cambridge Biotech HIV-1 Western Blot Kit, classifying all of the urine specimens as Western blot positive. There were no urine specimens that were negative or indeterminate in this population.

Table E
Frequency of Virus Specific Bands and Interpretation of Results of Urine Specimens from Non-AIDS HIV-1 Positive Populations
(N=533[a])

Urine EIA	Urine WB		Frequency of Virus Specific Bands[b]								
		N	p17	p24	p31	gp41	p51	p55	p66	gp120	gp160
RR[d]	Pos[f]	533	136	301	319	462	317	118	433	512	533[e]
N=533	Ind	0	0	0	0	0	0	0	0	0	0

[a] Two hundred and sixty two (262) specimens from Study 1, 197 from Study 2, 74 from Study 3.
[b] Band patterns for negative specimens do not appear in this table. By definition, negative specimens show no reactivity.
[d] RR indicates repeatedly reactive in the Calypte Biomedical HIV-1 Urine EIA.
[e] Fifty (50) of the 533 urine specimens had only a gp160 or gp120 and gp160 band (37 from Study 1, 8 from Study 2, 5 from Study 3).
[f] No intensity criterion associated with the LOW POSITIVE URINE CONTROL was used during this analysis.

Performance Using a Low Intensity Criterion

The performance of Western blot testing when using an intensity criterion for the gp160 band was evaluated by testing a subset of urine samples from known HIV-1 positive patients (Table F). One hundred and seventy-two (172) urine samples were retested. The results were interpreted by comparing the intensity of the gp160 band of the specimen with that of the LOW POSITIVE URINE CONTROL. The testing was performed, and results were interpreted at two different sites. A description of the known HIV-1 positive urine specimens that were retested and the results obtained at both sites are presented in Tables F and G.

The results show that the 2 sites read the same specimen as indeterminate. The sites differed on their interpretation for a second specimen (Site 1 read the sample as positive, Site 2 read the specimen as indeterminate). Therefore, the frequency of indeterminate results in a sample population of 172 HIV-1 EIA RR urine specimens ranged from 0.6% (1 of 172 specimens read at Site 1) to 1.2% (2 of 172 specimens read at Site 2).

The sensitivity of the Western blot using the intensity criterion associated with the LOW POSITIVE URINE CONTROL ranged from 98.3% (169/172) to 98.8% (170/172).

Table F
Description of HIV-1 Positive Specimens Retested
On The Cambridge Biotech HIV-1 Western Blot Kit

Study (N)	Study Group	Clinical Condition ((Samples Collected (N))	N Tested
1 (100)	HIV-1 Seropositive Women	AIDS (26) HIV-1 Symptomatic (38) HIV-1 Asymptomatic (36)	25[a] 37[b] 35[c]
2 (75)	AIDS Patients	AIDS (75)	75
Total (175)		175	172

[a] One of the 26 AIDS patient specimens had insufficient volume to test by Western blot.
[b] One of the 38 HIV-1 positive symptomatic patient specimens had insufficient volume to test by Western blot.
[c] One of the 36 HIV-1 positive asymptomatic patient specimens had insufficient volume to test by Western blot.

Table G
Western Blot Results for Known HIV-1
Positive Patients Using a gp160 Band Intensity Criterion

	No Intensity Criterion			Using gp160 Band Intensity Criterion Western Blot Results					
N	Prior[a] Western Blot Result			Site 1			Site 2[g]		
Tested	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg
172[b]	171	N/A	1[c]	170	1[d]	1[e]	169	2[f]	1[e]

[a] "Prior" indicates data from Table E: analysis without an intensity criterion.
[b] Three of the original 175 urine specimens had insufficient volume to test by Western blot.
[C] Specimen was borderline EIA repeatedly reactive (S/CO range 0.972 to 1.916), negative on Western blot in Table E.
[d] Specimen is +/- gp160/+gp120 only. The EIA testing associated with Western blot testing using Intensity Criterion was 1.284
S/CO. The specimen was previously urine Western blot false negative (Table E).
[e] Specimen was urine EIA non-reactive and false negative (S/CO=0.825).
[f] One Indeterminate was the specimen identified in footnote d.
[g] The blots were interpreted approximately 7 days after processing.

Specificity in Low Risk Groups

The specificity of the Cambridge Biotech HIV-1 Western Blot Kit was assessed by testing specimens from 515 EIA seronegative subjects at low risk for HIV-1 infection. The subjects were insurance applicants. Insurance applicants are presumed to be at low risk for HIV-1 infection. The results obtained from testing paired urine and serum specimens from low risk uninfected individuals by Western blot are provided in Table H.

The results show that in this low risk population, the specificity of the Cambridge Biotech HIV-1 Urine Western Blot was 100% (515 of 515 urine specimens were Western blot negative). There were no (0%) urine Western blot indeterminate or positive specimens.

Table H
Comparison of Cambridge Biotech HIV-1 Western Blot Results Using Urine and Paired Serum Specimens from Low Risk Populations
(N=515)

Serum					Urine			
Risk Group	N	Western Blot Results[c] Pos	Neg	Ind	EIA RR	Western Blot Results Pos[d]	Neg	Ind
Low[a]	200[b]	NT	NT	NT	0	0	200	0
	315	0	284	31	1	0	315	0

[a] Two hundred (200) specimens from Study 1, 315 specimens from Study 3.
[b] The 200 archived serum specimens (Study 1) were EIA NR and were not tested by Western blot.
[c] A licensed HIV-1 Western Blot Kit was used when testing serum specimens.
[d] No intensity criterion associated with the LOW POSITIVE URINE CONTROL was used during this analysis.

Frequency of Virus Specific Bands in Low Risk Groups

The frequency of virus specific bands and interpretation of results for urine specimens tested with the Cambridge Biotech HIV-1 Western Blot Kit for these low risk groups are presented in Table I.

The results show that in these populations of 515 low risk subjects, none of the urine specimens were positive or indeterminate when tested with the Cambridge Biotech HIV-1 Western Blot Kit. All 515 serum specimens were EIA non reactive; 200 serum specimens were not tested by Western blot; of 315 serum specimens that were tested by Western blot, 284 were negative, 31 were indeterminate, and none were positive. All 515 urine specimens were identified as Western blot negative, including the one urine specimen that was EIA initially reactive. This demonstrates the high specificity of the Cambridge Biotech HIV-1 Western Blot Kit for urine in low risk populations.

Table I
Frequency of Virus Specific Bands and Interpretation of Results of
Urine Specimens from Low Risk Populations
Tested by the Cambridge Biotech HIV-1 Western Blot Kit
(N=515[a])

Urine EIA	**Urine WB**		**Frequency of Virus Specific Bands[b]**								
		N	p17	p24	p31	gp41	p51	p55	p66	gp120	gp160
NR[c]	Pos	0	0	0	0	0	0	0	0	0	0
N=514	Ind	0	0	0	0	0	0	0	0	0	0
RR[d]	Pos	0	0	0	0	0	0	0	0	0	0
N=1[e]	Ind	0	0	0	0	0	0	0	0	0	0

[a] Two hundred (200) specimens from Study 1, 315 specimens from Study 3.
[b] Band patterns for negative specimens do not appear in this table. By definition, negative specimens show no reactivity.
[c] NR indicates non-reactive in the Calypte Biomedical HIV-1 Urine EIA.
[d] RR indicates repeatedly reactive in the Calypte Biomedical HIV-1 Urine EIA.
[e] The one urine specimen initially reactive in the EIA was not repeat tested.

Frequency of Virus Specific Bands in Other Groups

Two additional special studies of paired urine and serum specimens were collected for evaluation by Western blot. The first evaluation involved Western blot testing of urine specimens paired to serum EIA non-reactive Western blot indeterminate specimens (N=109). The purpose of the study was to demonstrate the specificity of the urine Western blot for those samples that are repeatedly reactive on the urine EIA. The results are shown in Table J.

In this evaluation, 4 of 109 (3.7%) urine specimens paired to serum Western blot indeterminate specimens were urine Western blot indeterminate. None were positive. One hundred and five (105) of 109 (96.3%) were negative. These results show the specificity of the Cambridge Biotech HIV-1 Western Blot Kit for samples from uninfected individuals who are repeatedly reactive (false positive) on the urine EIA.

Table J
Frequency of Virus Specific Bands and Interpretation of Results of
Urine Specimens Paired to Serum Western Blot Indeterminate Specimens from Uninfected Individuals
Tested by the Cambridge Biotech HIV-1 Western Blot Kit
(N=109[a])

Urine EIA	Urine WB		Frequency of Virus Specific Bands								
		N[d]	p17	p24	p31	gp41	p51	p55	p66	gp120	gp160
NR[b]	Pos	0	0	0	0	0	0	0	0	0	0
N=66	Ind	1	0	0	0	0	0	0	0	1	0
RR[c]	Pos[e]	0	0	0	0	0	0	0	0	0	0
N=43	Ind	3	0	2	0	0	0	0	1	0	0

[a] All 109 specimens were from Study 3.
[b] NR indicated non-reactive in the Calypte Biomedical HIV-1 Urine EIA.
[c] RR indicates repeatedly reactive in the Calypte Biomedical HIV-1 Urine EIA.
[d] Includes specimens with non-viral bands.
[e] No intensity criterion associated with the LOW POSITIVE URINE CONTROL was used during this analysis.

The second evaluation involved urine Western blot testing of urine EIA repeatedly reactive specimens paired to serum EIA non-reactive specimens from uninfected individuals (N=114). The purpose of this study was to assess the utility of the urine Western blot for individuals who are not infected but whose urine specimens are repeatedly reactive using the HIV-1 EIA. The frequency of virus specific bands in each group of urine specimens tested is demonstrated in the Table K.

Of the 114 urine EIA repeatedly reactive specimens paired to serum EIA non-reactive specimens, 109 of 114 (95.6%) were Western blot negative, 5 of 114 (4.4%) were Western blot indeterminate and none were positive. This demonstrates the ability of the Cambridge Biotech HIV-1 Western Blot Kit to resolve urine EIA repeatedly reactive specimens from uninfected individuals as negative or indeterminate.

Table K
Frequency of Virus Specific Bands and Interpretation of Urine EIA False Positive Urine Specimens Tested by the Cambridge Biotech HIV-1 Western Blot Kit
(N=114[a])

Urine EIA	Urine WB		Frequency of Virus Specific Bands								
		N	p17	p24	p31	gp41	p51	p55	p66	gp120	gp160
RR[b]	Pos	0	0	0	0	0	0	0	0	0	0
N=114	Ind	5	0	5	0	0	0	0	0	0	0

[a] All 114 specimens were from Study 3.
[B] RR indicates repeatedly reactive in the Calypte Biomedical HIV-1 Urine EIA.

Specificity in Subjects with Medical and Other Conditions

The specificity of the Cambridge Biotech HIV-1 Western Blot Kit was evaluated using urine from individuals with medical conditions unrelated to HIV-1 and from individuals with potential interfering substances in their urine. The results of testing urine specimens from these individuals with the Cambridge Biotech HIV-1 Urine Western Blot Kit are provided in Table L.

The results obtained from testing urine specimens collected from patients with diseases and potentially interfering substances showed that 2 urine specimens were urine EIA repeatedly reactive and Western blot positive and were paired to serum EIA repeatedly reactive and Western blot positive specimens.

For subjects with non-HIV-1 medical conditions, the specificity of the urine Western blot for EIA repeatedly reactive urine specimens in this study was 90.1% (100/111).

Table L
Comparison of Cambridge Biotech HIV-1 Western Blot Results Using Urine and Paired Serum in Populations with Disease and Potentially Interfering Substances
(N=281)

		Urine EIA Results		Urine Western Blot Results		
Group	**N**	**RR**	**NR**	**Pos[j]**	**Neg**	**Ind**
Autoimmune[a]	25	7	18	0	25	0
Kidney/Liver[b]	59	32	27	0	55	4
STD[c]	37	5	32	0	37	0
Urine Cond.[d]	47	22	25	1	45	1
Pregnant[e]	63	25	38	1	59	3
Neoplasms[f]	35	17	18	0	35	0
Multiple Transfusions[g]	13	5	8	0	10	3
Multiparous[h]	2	0	2	0	2	0
Total	281	113	168	2[m]	268	11[k]

[a] Twenty (20) specimens from Study 1, 5 specimens from Study 3.
[B] Twenty (20) specimens from Study 1, 39 specimens from Study 3.
[c] STD = Sexually transmitted disease. Twenty two (22) specimens from Study 1, 15 specimens from Study 3.
[d] Twenty (20) specimens from Study 1, 27 specimens from Study 3.
[e] Sixty three (63) specimens from Study 3.
[f] Thirty five (35) specimens from Study 3.
[g] One (1) specimen from Study 2, 12 specimens from Study 3.
[h] Two (2) specimens from Study 3.
[j] No intensity criterion associated with the LOW POSITIVE URINE CONTROL was used during this analysis.
[k] These specimens were all urine EIA repeatedly reactive.
[m] These two specimens were true serum positive as confirmed.

Dilution of Paired Urine and Serum Specimens

Ten (10) paired urine and serum specimens from HIV-1 positive individuals were tested in a dilution study using the Cambridge Biotech HIV-1 Western Blot Kit. The urine specimens were tested at dilutions of 1:2, 1:10, 1:50, 1:500 and 1:1,000. The serum specimens were tested at dilutions of 1:101, 1:1,000, 1:5,000, 1:25,000 and 1:50,000. The urine specimens were tested according to the urine procedure. The serum specimens were tested according to the manufacturer's package insert instructions. The major viral bands (gp160, gp120, gp41 and p24) were observed for presence or absence on Western blot at each dilution. The results are reported as the last dilution at which a band is visible on Western blot without comparison to an intensity criterion.

A side by side comparison of the urine and serum dilutions are presented in Table M. The comparison shows that the gp160 band can be observed at a higher dilution than the gp120, gp41 or p24 bands for both urine and serum specimens tested in dilution series.

The difference in the analytical sensitivity between the licensed Serum Western Blot Kit and the Urine Western Blot Kit for antibodies to different proteins ranged from a ratio of greater than 50 to greater than 25,000.

Table M
Comparison of Dilutions of HIV-1 Positive Paired Urine & Serum Specimens Tested on Cambridge Biotech HIV-1 Western Blot Kit
Last Dilution At Which Major Viral Band is Present
Major Viral Bands^

No.	Specimen No.	Clinical Classification*	gp160		gp120		gp41		p24	
			Urine	Serum	Urine	Serum	Urine	Serum	Urine	Serum
1	SP00000604	AIDS	1:500	>1:50,000	1:10	>1:50,000	1:10	>1:50,000	-	-
2	SP00000606	AIDS	1:50	>1:50,000	1:2	>1:50,000	1:2	>1:50,000	-	1:1,000
3	SP00001709	HIV-1 Symptomatic	1:10	>1:50,000	-	>1:50,000	-	>1:50,000	-	1:10,000
4	SP00001752	HIV-1 Symptomatic	1:50	>1:50,000	1:2	>1:50,000	1:2	>1:50,000	1:10	>1:50,000
5	SP00001783	HIV-1 Asymptomatic	1:50	>1:50,000	1:2	>1:50,000	-	1:10,000	-	1:101
6	CL1	F-TL	1:50	>1:50,000	-	>1:50,000	-	>1:50,000	-	>1:50,000
7	CL35	F-TL	1:10	>1:50,000	-	1:25,000	-	1:10,000	-	1:10,000
8	CL83	F-TL	1:1,000	>1:50,000	1:50	>1:50,000	1:10	>1:50,000	>1:1,000	>1:50,000
9	175	F-TZ	1:500	>1:50,000	1:50	>1:50,000	1:10	>1:50,000	1:1,000	>1:50,000
10	3.030751	F-Abidj	1:10	>1:50,000	-	>1:50,000	-	1:25,000	1:10	>1:50,000

* **F-TL = Foreign specimen from Thailand**
* **F-TZ = Foreign specimen from Tanzania**
• **F-Abidj = Foreign specimen from Abidjan**
^ **(-) indicates these bands were not present for the original serum specimen diluted 1:101 according to the serum Western Blot procedure and for the original urine specimen diluted 1:2 according to the Western Blot procedure for urine.**

Reproducibility

The reproducibility of the Cambridge Biotech HIV-1 Western Blot Kit was evaluated by testing a panel of urine specimens at three (3) geographically separate sites. A panel of 12 specimens of defined viral reactivity was provided to the three sites for evaluation.

The panel consisted of specimens strongly reactive and weakly reactive for antibodies to HIV-1 and specimens non-reactive for antibodies to HIV-1. Each panel member was tested in duplicate on three different lots of the Cambridge Biotech HIV-1 Western Blot Kit. The testing was performed by at least two different operators at each of 3 sites over multiple days.

In addition to the 12 specimens, a high positive urine control, low positive urine control and a negative urine control were provided for testing with each kit.

The results of this analysis demonstrate the reproducibility of the Cambridge Biotech HIV-1 Western blot for urine specimens with HIV-1 antibody activity to the gp160 viral gene product at the limit of visual detection. The combined results of testing are provided in Table N.

Table N
Number of Replicates of Western Blots With Reactive Bands
(% Reactive Replicates)

Spec/ Res[a]	# of Reps[h]	p17	p24	p31	gp41	p51	p55	p66	gp120	gp160
HPC[b]	24	23 (96)	24 (100)	24 (100)	24 (100)	15 (63)	13 (54)	24 (100)	24 (100)	24 (100)
LPC[c]	24	3 (13)	24 (100)	23 (96)	17 (71)	6 (25)	0 (0)	21 (88)	24 (100)	24 (100)
NC[d]	24	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1 Neg[e]	48	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
2 Neg[c]	48	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
3 S. Pos[f]	48	16 (33)	2 (4)	48 (100)	20 (42)	28 (58)	18 (38)	48 (100)	48 (100)	48 (100)
4 S. Pos[f]	40	40 (100)	40 (100)	40 (100)	40 (100)	34 (85)	22 (55)	40 (100)	40 (100)	40 (100)
5 W. Pos[g]	40	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	14 (35)	0 (0)	40 (100)	40 (100)
6 W. Pos[g]	40	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	40 (100)	40 (100)
7 W. Pos[g]	48	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	28 (58)	48 (100)	48 (100)
8 W. Pos[g]	48	0 (0)	46 (96)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	48 (100)	48 (100)
9 W. Pos[g]	48	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4)	48 (100)	48 (100)
10 W. Pos[g]	48	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	48 (100)	48 (100)
11 W. Pos[g]	48	0 (0)	24 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	48 (100)	48 (100)
12 W. Pos[g]	48	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	48 (100)	48 (100)

[a] Spec/Res=Specimen ID#/Expected Result
[b] HPC=High Positive Urine Control
[c] LPC=Low Positive Urine Control
[d] NC=Negative Urine Control
[e] Neg=Negative Specimen
[f] S Pos=Strong Positive Specimen
[g] W Pos=Weak Positive Specimen
[h] Reps=Replicates

VII. Conclusions Drawn from the Studies

The studies conducted with Calypte Biomedical Corporation's Cambridge Biotech HIV-1 Western Blot product leading to original approval on 5/28/98 as a license supplement (reference number 95-1588) are sufficient to permit approval of the PMA and continued marketing of the product. No changes in manufacturing processes, facilities, or quality control specifications are being made from the previously licensed product. This PMA reflects only a change in the packaging and labeling of the product.

VIII. Panel Recommendation

This PMA submission was not referred to an FDA advisory committee for review or recommendation.

IX. CBER Decision on Application

FDA issued an approval order on _____.

X. Approval Specifications

Directions for Use:	See the product labeling.
Conditions of Approval:	None.
Postapproval Requirements and Restrictions:	None.