Summary of Safety and Effectiveness
BP000009 - Calypte™ HIV-1 Urine EIA

I. General Information

Device Generic Name:	Enzyme Immunoassay for the Detection of Antibodies to Human Immunodeficiency Virus (Type 1 (HIV-1) in Urine
Device Trade Name:	Calypte™ HIV-1 Urine EIA
Applicant's Name and Address:	Calypte Biomedical Corporation 1265 Harbor Bay Parkway Alameda, CA 94502
PMA Number:	BP000009
Date of Panel Recommendation:	None
Date of Good Manufacturing Inspection:	November 13 – 17, 2000

II. Indications for Use

The Calypte™ HIV-1 Urine EIA test is an enzyme immunoassay for the in vitro detection of antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) in urine. The test is intended for use in professional laboratory settings as an aid in clinical diagnosis of HIV infection. Before a determination of HIV-1 status can be made, urine specimens that are repeatedly reactive using this test should be further tested using the additional, more specific Calypte Biomedical Cambridge Biotech HIV-1 Urine Western blot kit.

III. Background/Device Description

Summary and Explanation of the Test

Acquired Immunodeficiency Syndrome (AIDS) is the result of the progressive loss of immunocompetence following infection with the Human Immunodeficiency Virus (HIV). Individuals exposed to HIV may experience an initial acute phase illness characterized by flu-like symptoms. The acute phase is followed by a putative latency period of varying length, culminating in the onset of the symptoms of opportunistic infections which characterize AIDS. Most individuals infected by HIV develop antibodies to the major structural proteins of HIV. Detection of these antibodies in blood has long been considered

prerequisite for the diagnosis of AIDS. Recent evidence also demonstrates the detection of antibodies to HIV in the urine of HIV infected individuals.

There are several safety advantages to using urine specimens compared with the use of blood. Infectious Human Immunodeficiency Virus is unlikely to be present in urine, and urine can be collected by a non-invasive procedure. The use of urine eliminates the risk of accidental needlestick exposure to bloodborne pathogens during the collection of specimens. An HIV-1 urine antibody test may facilitate surveillance for HIV infection. With the availability of an HIV-1 Western blot (Calypte Biomedical Corporation's Cambridge Biotech HIV-1 Western blot kit) approved for use with urine specimens, urine specimens that are repeatedly reactive in the HIV-1 Urine EIA may be further tested for the presence of HIV-1 antibodies.

Principles of the Procedure

The Calypte™ HIV-1 Urine EIA is an enzyme immunoassay which utilizes a recombinant envelope protein of HIV-1 to detect the presence of antibodies to HIV-1 in human urine. The recombinant gp160 envelope protein is adsorbed onto the wells of a microwell plate. Urine specimens or urine controls which may contain antibodies to HIV-1, along with a sample buffer, are added to the wells and incubated. If antibodies to the HIV-1 envelope protein are present in the specimen, they will bind to the antigen coated on the well. The sample buffer significantly reduces the non-specific binding of antibodies and other proteins to the well. A wash step removes any unbound material. Then, a conjugate consisting of alkaline phosphatase chemically bound to goat anti-human immunoglobulin is added to each well and allowed to incubate. The conjugate will bind to HIV-1 antibodies which are bound to the immobilized antigen. A wash step removes any unbound conjugate. The substrate for the enzyme, p-nitrophenylphosphate (p-NPP), is added to all wells and incubated. If antibodies to HIV-1 are present in the specimen, the enzyme will cause the color to change from colorless to yellow. The intensity of the color is proportional to the amount of HIV-1 antibody present in the test specimen or control. The reaction is terminated by the addition of a stop solution containing ethylenediaminetetraacetic acid (EDTA). The absorbance values are determined spectrophotometrically with a plate reader at a peak wavelength of 405 nm.

Using the positive control and negative calibrator included with the test kit, two positive control wells and three negative calibrator wells are tested with each plate or partial plate of specimens. A specimen is determined to be either reactive or non-reactive by comparing its absorbance value to a cutoff value which is calculated by adding the mean absorbance value of the negative calibrators to a value of 0.180.

Reagents

Label	Component	Contents
1	**RECOMBINANT gp160 HIV-1 ANTIGEN COATED MICROWELL PLATE** One plate holds 6 - 2x8-well strips (96 wells), with adsorbed recombinant gp160 and 0.1% sodium azide. Plates are provided in resealable foil pouches with desiccant.	2 Plates (192 wells) or 5 Plates (480 wells)
2	**SAMPLE BUFFER** Contains buffered animal (bovine, caprine, equine) sera and 0.08% sodium azide as preservative.	1 Bottle (25 mL)
3	**NEGATIVE CALIBRATOR** Contains human urine negative for antibodies to HIV-1 and 0.1% sodium azide as preservative. Non-reactive for HBsAg.	1 Bottle (9 mL)
4	**POSITIVE CONTROL** Contains human urine positive for antibodies to HIV-1 and 0.1% sodium azide as preservative. Non-reactive for HBsAg.	1 Bottle (6 mL)
5	**CONJUGATE CONCENTRATE** Alkaline phosphatase labeled goat anti-human immunoglobulin in tris-buffered saline with bovine serum albumin and 0.04% sodium azide as preservative.	1 Vial (400 µL)
6	**CONJUGATE DILUENT** Contains tris-buffered saline with goat serum and 0.1% sodium azide as preservative.	1 Bottle (100 mL)
7	**10X WASH SOLUTION** Contains a concentrate of tris-buffered saline with NP-40 and 1.0% sodium azide as preservative.	1 Bottle (450 mL) or 2 Bottles (450 mL) provided separately
8	**SUBSTRATE TABLETS** 5 mg tablets of p-nitrophenylphosphate (p-NPP) in foil packets.	15 Tablets or 25 Tablets
9	**SUBSTRATE DILUENT** Contains diethanolamine buffer with magnesium chloride and 0.1% sodium azide as preservative.	1 Bottle (150 mL)
10	**STOP SOLUTION** Contains ethylenediaminetetraacetic acid (EDTA).	1 Bottle (35 mL)

PLATE SEALERS Twenty-five sealers per package.	1 Package or 2 Packages

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 the supplemental urine HIV-1 Western Blot product (Calypte Biomedical Corporation's Cambridge Biotech HIV-1 Western blot). Several products are available to detect antibodies to HIV in human serum, plasma, dried blood spots and oral fluid.

V. Marketing History

The Calypte HIV-1 Urine EIA product was approved by the FDA as a licensed biologic on August 6, 1996 and has been in commercial distribution in the United States since that date. The product has also been distributed outside of the United States in Brazil, Canada, China, Egypt, England, France, India, Italy, Ivory Coast, Japan, Malaysia, South Africa, South Korea, Spain, and Uganda.

VI. Summary of Studies

A summary of the original studies supporting the efficacy of the device are detailed in the product package. Data generated to support the manufacture of the Calypte HIV-1 Urine EIA in the Alameda facility consisted of nonclinical laboratory studies (stability studies) and several clinical studies to demonstrate that product produced in the Alameda, California facility performed in a comparable manner to product manufactured in the previously approved Berkeley, California facility.

Nonclinical Laboratory Studies

Nonclinical laboratory studies reported in this submission include stability studies supporting expiration dating of all kit components for at least 15 months from the their date of manufacture.

Clinical Studies

The PMA submission describes the relocation of manufacturing facilities from the currently licensed Berkeley, California facility to a new facility in Alameda, California. The results of the clinical studies conducted to demonstrate that

product manufactured in the new Alameda, CA facility performed comparably to product manufactured in the currently licensed Berkeley, CA facility are reported in the PMA. The studies were conducted with three consecutively manufactured Alameda kit lots which met current lot release specifications and two Berkeley kit lots comparing the sensitivity, specificity, reproducibility and analytical sensitivity of HIV-1 Urine EIA Kits. Protocols describing the selection of specimens, preparation of the specimens and the testing procedures for comparing the sensitivity, specificity and reproducibility of results were submitted to FDA prior to initiation of the study.

Comparability was assessed in three ways: 1) by testing a panel of 455 HIV-1 antibody positive and HIV-1 antibody negative urine specimens on multiple kit lots produced at each facility (Comparability Study); 2) by testing the reproducibility of a panel of 11 HIV-1 antibody positive and HIV-1 antibody negative urine specimens over multiple days on multiple kit lots produced at each facility (Reproducibility Study); and 3) by testing two-fold dilutions of 10 HIV-1 antibody positive urine specimens on multiple kit lots produced at each facility (Dilution Study).

The comparability, reproducibility and dilution studies were performed at three separate licensed clinical laboratories. Each of the laboratories was provided with aliquots of the same urine specimens, including the two-fold dilutions of HIV-1 antibody positive urine specimens. Each laboratory used identical testing protocols when testing the specimens.

For the Comparability and Dilution studies specimens were tested on the 5 kit lots by the same operator within a given day. For the Reproducibility study, specimens were tested according to a schedule provided in the study protocol. This schedule involved testing over multiple days by multiple operators.

Comparability Study

All of the specimens included in the Comparability Panel were tested on all 5 kit lots at each of the 3 participating laboratories. Specimens were assayed on all 5 lots within the same day. The specimens were tested once on each kit lot. EIA reactive specimens were not repeat tested in duplicate and were not tested by Western blot.

Analysis of All Comparability Specimens

The results of specimens tested in the Comparability Study were analyzed in several ways. First, the reactivity of the specimens from the 3 labs was analyzed by individual kit lot. Then the results were analyzed by comparing the results obtained for each Berkeley kit lot to the results obtained for the 3 Alameda kit lots combined. The results show that the reactivity observed for the combined results from the 3 laboratories for the comparability specimens tested on each individual

lot were similar across the 5 lots. The reactivity observed for the Alameda kit lots of 20.2%, 18.9% and 19.3% fell within the reactivity observed for the Berkeley kit lots of 17.9% and 21.2%.

Test Agreement for Berkeley and Alameda Kit Lots

A comparison of the combined test results for the 3 Alameda kit lots for all 455 specimens with each of the two Berkeley kit lots was conducted. The percentages of outcomes which agree (i.e., Agreement) between the paired data sets were calculated along with corresponding exact binomial 95% confidence interval (lower interval, upper interval).

Berkeley Lot 1 versus all Alameda lots: Agreement = 95.7% (95.0%, 96.3%)
Berkeley Lot 2 versus all Alameda lots: Agreement = 95.6% (95.0%, 96.2%)

The results obtained showed that the number of positive test results for kit lots produced at Alameda was similar to the number of positive test results for kit lots produced at Berkeley. The agreement between results obtained for Berkeley kit lots 1 and 2 and all kit lots produced at Alameda were similar giving 95.7% and 95.6% agreement, respectively. The 95% confidence limits for comparison of lots from both manufacturing facilities were similar, ranging from 95.0% to 96.3%.

Comparison of Test Sensitivity for Berkeley and Alameda Kit Lots

Kit lots manufactured at Berkeley and at Alameda were compared for their ability to detect antibody in urine specimens from patients known to be positive for antibodies to HIV-1.

The test results obtained from all AIDS, HIV-1 symptomatic, and HIV-1 asymptomatic specimens, including weakly reactive specimens, were similar for all Alameda and Berkeley kit lots. The number of positive observations was similar for Alameda and Berkeley kit lots. The sensitivity computed for AIDS, HIV-1 symptomatic and HIV-1 asymptomatic specimens demonstrated that the combined lots from the Alameda facility (95.4%) had a sensitivity between that of Berkeley lot 1 (94.5%) and lot 2 (96.7%) confidence intervals.

Reactivity Rates for Berkeley and Alameda Kit Lots for HIV-1 Antibody Positive Specimens

The reactivity rates for specimens from patients with AIDS and from patients with or without symptoms of HIV infection were evaluated. The reactivity rates for specimens tested with Alameda produced kit lots (94.5% to 96.7%) were observed to fall within the ranges of reactivity for Berkeley produced kit lots (94.5% and 96.7%). The reactivity rates for each specimen category for combined kit lot results were similar between Berkeley and Alameda produced kit lots. Using Fischer's Exact analysis, no significant differences in reactivity rates between Berkeley and Alameda kit lots were observed for specimens from AIDS,

HIV-1 symptomatic or HIV-1 asymptomatic subjects or from all categories combined. These analyses show that Alameda kit lots demonstrate equivalent sensitivity to Berkeley kit lots for these specimens.

Reactivity Rates for Berkeley and Alameda Kit Lots for Low Risk Specimens

The reactivity rates for specimens from subjects at low risk of HIV-1 infection with Alameda produced kit lots were observed to be within the range of the rates observed for Berkeley produced kit lots, except for one Alameda lot. The reactivity rate for this lot was slightly higher than the two Berkeley lots (3.1% for Alameda vs. 1.0%, 2.4% for Berkeley). The reactivity rate for specimens from low risk subjects was 1.73% for the combined Berkeley kit lots and 2.44% for the combined Alameda kit lots. These rates are not significantly different (i.e., p was >0.05) when tested by Chi square analysis.

These specificity rates (test results from 3 labs on 260 specimens) are comparable to the specificity rates reported for initially reactive specimens in the performance section of the Calypte™ HIV-1 Urine EIA package insert. The rates in the package insert ranged between 1.1% and 2.0% for 7,082 randomly collected urine specimens from low risk subjects.

Reactivity Rates for Berkeley and Alameda Kit Lots for High Risk Specimens

There were 80 urine specimens from subjects considered at high risk of HIV infection included in the study. Of the 80 specimens, 29 were from subjects visiting an STD clinic, 26 were from seronegative partners of HIV-1 seropositive individuals, 21 were individuals that had multiple sexual partners, 3 were injection drug users, and one (1) was a commercial sex worker. The reactivity rates for specimens from subjects at high risk for HIV-1 infection were tested. The rates for specimen tested with Alameda produced kit lots (5.4% to 7.1%) produced rates that were comparable to Berkeley produced kit lots (4.2% to 8.3%).

The reactivity rates for all specimen categories tested with Berkeley and Alameda kit lots were similar. None of the rates between Berkeley and Alameda kit lots were significantly different (i.e., p>0.05) using a Fischer's Exact analysis (6.25% vs.6.81%, respectively).

Reactivity Rates for Berkeley and Alameda Kit Lots for False Positive Specimens

A subpopulation of 20 of the 80 specimens from subjects at high risk of HIV infection were known to give false positive results in the HIV-1 Urine EIA from prior testing. Of the 20 false positives, 9 specimens were from subjects visiting

an STD clinic and 11 were from subjects with other risk factors. The percent reactivity observed for these prior false positive specimens tested in the HIV-1 Urine EIA for the combined Berkeley lots (19.2%) and the combined Alameda lots (21.7%) are similar.

Reactivity Rates for Berkeley and Alameda Kit Lots for Subjects with Autoimmune Disease, Urinary Tract Infections and Neoplasms

Specimens from patients with autoimmune disease (n=11), urinary tract infections (n=21), and neoplasms (n=22), were evaluated by reactivity for each individual kit lot. The total reactivity rates for specimens from patients with autoimmune disease, urinary tract infections, and neoplasms ranged from 33.3% to 45.1% for Berkeley, and from 35.8% to 37.0% for Alameda kit lots. These data suggest that these Alameda produced kit lots are at least equivalent to Berkeley produced kit lots for this population of disease state specimens tested. The HIV-1 Urine EIA reactivity rates for all specimen categories for Berkeley compared to Alameda kit lots were similar (39.2% and 36.2%, respectively). None of the reactivity rates between Berkeley and Alameda kit lots were significantly different (i.e., $p>0.05$) using a Fischer's Exact analysis.

Reproducibility Study

The Reproducibility Study was designed to evaluate lot-to-lot, within-plate, plate-to-plate (within-day), day-to-day, operator-to-operator and laboratory-to-laboratory reproducibility of the 5 different lots of kits, 2 Berkeley and 3 Alameda. The reproducibility study consisted of testing 11 specimens in 2 separate runs (assays) per day on each of 12 different days.

This analysis indicates that the S/CO for the specimens tested on lots manufactured at the two facilities did not differ after adjustment for lab, lot, specimens, and pouch for specimens determined to be positive or negative. In addition, the variances for these specimens were within the performance characteristics specified in the package insert of the HIV-1 Urine EIA.

Dilution Study

The purpose of the Dilution Study was to evaluate the sensitivities of the HIV-1 Urine EIA Kits manufactured at the Alameda facility in comparison to HIV-1 Urine EIA kits manufactured at the Berkeley facility using diluted specimens. In this study, sensitivity was determined by testing a series of dilutions of 10 HIV-1 antibody positive specimens and comparing the endpoints for each specimen across lots. The endpoint was defined as the last dilution at which the specimen was reactive in the HIV-1 Urine EIA.

Dilution specimens demonstrated similar sigmoidal dose response curves for strongly reactive specimens or parabolic dose response curves for weakly reactive

specimens with both Berkeley and Alameda kit lots. The linear range of reactivity for all kit lots was from approximately 2 S/CO to 9 S/CO. The similarities of all dose response curves for all specimens tested indicate that the Berkeley and Alameda kit lots are producing similar reactivity responses. The endpoints were within a two-fold dilution across all labs and all lots for all specimens.

Each Berkeley kit lot was compared to all three Alameda kit lots for each dilution tested in two by two tables to determine the agreement between Alameda and Berkeley manufactured kit lots. The number of positive observations for kit lots produced by the Berkeley lots and the Alameda lots was similar. The rates of agreement computed for the two Berkeley lots were 97.1% and 98.5%, respectively. These rates of reactivity agreement between Berkeley and Alameda kit lots do not suggest a difference between kit lots manufactured at the two facilities since the confidence limits overlap.

VII. Conclusions Drawn from the Studies

Urine specimens were tested to compare the sensitivity, specificity and reproducibility of HIV-1 Urine EIA kits manufactured at the Berkeley facility with HIV-1 Urine EIA kits manufactured at the Alameda facility. The same two lots of Berkeley produced HIV-1 Urine EIA test kits and three lots produced at the Alameda facility were used in all of the studies conducted at all of the laboratories, using well defined protocols.

In a comparability study, 455 specimens known to have specific HIV-1 reactivity (positive, negative, weakly) and non-specific reactivity were tested using the test kit lots. The data from these tests show that both Berkeley and Alameda kit lots identified a similar number of initial reactive specimens. In addition, the non-specific reactivity rates for selected urine false positive specimens, and urine specimens from subjects with urinary tract infections, autoimmune diseases and neoplasms were not significantly different for lots produced at the two facilities. A reactivity frequency analysis of the S/CO values for specimens from individuals at low risk of HIV infection and individuals known to be HIV positive indicated that Berkeley and Alameda kit lots gave similar reactivity curves demonstrating that the test kits are calibrated in a similar manner.

A reproducibility study was conducted to determine whether there was a difference among test kit lots for the S/CO values of a panel of 11 specimens. Testing occurred in three laboratories over 12 testing days. The results of these tests indicated that the S/CO values obtained from kit lots produced at the two facilities pouch for specimens determined to be positive or negative did not differ after adjustment for lab, lot, and specimen. In addition, the variation observed

was equivalent to or less than the variation described in the Performance Characteristics section of the current HIV-1 Urine EIA package insert.

In the urine dilution study, in order to determine a difference in test kit lot detection of diminishing concentrations of antibody in urine, HIV-1 antibody positive urine specimens were diluted in two fold dilutions to end-point. In all cases Alameda kit lots when analyzed, either combined or separately, gave equivalent reactivity to the Berkeley kit lots with all dilution endpoints being within a two-fold dilution across all labs and all lots for all specimens.

The studies reported in the PMA submission demonstrate that product produced in the new Alameda, California facility is comparable in performance to product produced in the previously approved Berkeley, California facility.

VIII. Panel Recommendation

The 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 _____.

The applicant's Alameda, California manufacturing facility was inspected on November 13 – 17, 2000 and was found to be in compliance with the device Quality System (Good Manufacturing Practices) regulations.

X. Approval Specifications

Directions for Use:	See the product labeling.
Conditions of Approval:	See approval order.
Postapproval Requirements and Restrictions:	See approval order.