

MAXIM BIOMEDICAL, INC.

HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1) CAMBRIDGE BIOTECH HIV-1 URINE WESTERN BLOT KIT For Detection of Antibodies to HIV-1 (Cat. No. 98078)

NAME AND INTENDED 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 urine specimens. This more specific assay is used as a supplemental test with urine specimens that tested repeatedly reactive using a screening procedure (Maxim HIV-1 Urine EIA Kit). The test is intended for use in professional laboratory settings as an aid in clinical diagnosis of HIV infection.

Conditions for Use:

1. Only those urine specimens that are repeatedly reactive using the Maxim HIV-1 Urine EIA Kit are to be further tested using this product, the Cambridge Biotech HIV-1 Urine Western Blot Kit.
2. Report test results only to the ordering physician or someone under the supervision of the ordering physician. Report test results to test subjects based on complete EIA and Western blot testing, as necessary.
3. This test using urine specimens is not to be used for testing potential blood donors.

NOTE: See "Warnings," "Precautions," "Reporting of Results," "Performance Characteristics" sections of this package insert and the Subject Information Brochure for information on:

THE SPECIFICITY OF CAMBRIDGE BIOTECH HIV-1 WESTERN BLOT TESTING USING URINE SPECIMENS IS REDUCED FOR HIGH RISK POPULATIONS COMPARED WITH TESTING USING SERUM OR PLASMA. FALSE POSITIVES MAY OCCUR MORE OFTEN WHEN TESTING URINE SPECIMENS.

SUMMARY AND EXPLANATION OF TEST

The Enzyme-Linked Immunosorbent Blot Technique ("Western Blot")^{1,2} 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).^{2,3} 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 Urine Western Blot Kit, when used as directed in this insert, will detect antibodies^{4, 5} to HIV-1 when present in human 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.⁴

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.¹⁷

CHEMICAL AND BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The Cambridge Biotech HIV-1 Urine Western Blot Kit is manufactured by Maxim Biomedical from HIV-1 propagated in an H9/HTLV-III B T-Lymphocyte cell line.³ 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

Reagents for the Cambridge Biotech HIV-1 Urine Western Blot Kit include:

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.

CAUTIONS

1. HANDLE ASSAY SPECIMENS, STRIPS, AND REACTIVE AND NON-REACTIVE CONTROLS AS IF CAPABLE OF TRANSMITTING AN INFECTIOUS AGENT. Inactivated HIV-1 antigen has been electrophoresed and transferred onto nitrocellulose. The NEGATIVE, LOW and HIGH POSITIVE URINE CONTROLS have been inactivated by heat treatment. A serum sample from the source of the urine used to manufacture the URINE CONTROLS was shown to be non-reactive for hepatitis B surface antigen and hepatitis C antibodies. However, no known test method can offer assurance that products derived from human source materials will not transmit infectious agents. Therefore, these components must be handled as if they are capable of transmitting infectious agents.
2. Do not pipet by mouth.
3. Wear disposable gloves throughout the procedure. Dispose of gloves as biohazard waste. Thoroughly wash hands after handling test reagents.
4. Wipe spills promptly with a 0.5% sodium hypochlorite solution (1/10 dilution of liquid household bleach). Contaminated materials should be disposed of as biohazard waste.
5. Dispose of all specimens and materials used in the Cambridge Biotech HIV-1 Urine Western Blot Kit procedure as biohazard waste. The recommended method of disposal is autoclaving for a minimum of 1 hour at 121°C. Disposable materials may be incinerated. Mix 9 volumes of liquid wastes with 1 volume of 5% sodium hypochlorite solution (liquid household bleach), allowing at least 60 minutes for disinfection.
6. Do not permit SUBSTRATE, especially 4-chloro-1-naphthol, to contact the skin. If contact occurs, flush with water.
7. The URINE CONTROLS contain sodium azide as a preservative. If these materials, either concentrated or diluted, are disposed of through a sink or other common plumbing systems, flush with generous amounts of water to prevent accumulation of potentially explosive compounds.
In addition, consult the manual guideline, "Safety Management No. CDC-22, 'Decontamination of Laboratory Sink Drains to Remove Azide Salts' " (Centers for Disease Control, Atlanta, GA, April 30, 1976).
8. Avoid use of metal instruments in contact with SUBSTRATE B and WORKING SUBSTRATE SOLUTION, since metals can cause reduction in H₂O₂.

PRECAUTIONS

WARNING:

- **The Cambridge Biotech HIV-1 Urine Western Blot kit has reduced sensitivity and reduced specificity using urine specimens, compared with using serum or plasma specimens.**
- **FDA has licensed this test kit for use with urine specimens only. Use of this licensed test kit with specimens other than those specifically approved for use with this test kit may result in inaccurate test results.**

1. DO NOT INTERCHANGE REAGENTS (e.g. urine controls) BETWEEN KIT LOTS.
2. Reagents may have expiration dating beyond the kit expiration dating. DO NOT USE REAGENTS BEYOND KIT EXPIRATION DATING. Kit expiration dating is printed on The outer box label.
3. Avoid contamination of reagents when opening and withdrawing aliquots from primary vials. Keep all reagents refrigerated (2-8°C) when not in use.
4. Do not interchange vial or bottle caps and stoppers; this will lead to cross contamination of reagents. Designate specific reservoirs for specific reagents.
5. Grossly contaminated specimens or strips may result in the development of dark spots on the strip which should not be interpreted. Careful attention must be given to the storage of specimens and kits to prevent this problem.
6. Shield WORKING SUBSTRATE SOLUTION from sunlight during preparation and use within 30 minutes of mixing.
7. Use reagent grade water (deionized water which is bacteria-free) to dilute reagents in order to avoid substances which may interfere with the assay.
8. Do not remove NITROCELLULOSE STRIPS from the storage tube until immediately before use. To prevent moisture from condensing inside the strip tube, open only AFTER the strips have reached room temperature (approximately 30 minutes). Close the tube immediately after removing strips for use.
9. Allow all kit reagents and materials to reach room temperature before use (approximately 30 minutes).
10. Use only the CONTROLS supplied with the kit for the analysis of urine specimens.
11. DO NOT CUT STRIPS. Narrower strips can lead to misinterpretation because strips may flip over in the incubation tray, or artifacts in the reaction zones may be mistaken for possible bands or may prevent recognition of positive bands.

12. Measure all reagents. Use extreme care and calibrated pipets with good quality tips when preparing WORKING CONJUGATE SOLUTIONS.
13. Discard bleach solution in trap prior to substrate preparation.

PREPARATION OF REAGENTS

NOTE: Allow reagents to reach room temperature before use (approximately 30 minutes). Consult table on p. 7 to determine the volumes of prepared reagents required for the number of strips being run.

1. DILUTED WASH BUFFER

- a. Dilute 1 volume of WASH BUFFER (20x) with 19 volumes of reagent grade water. Mix well.

DILUTED WASH BUFFER may be stored at room temperature (20-28°C) for 3 months.

2. WORKING BLOTTING BUFFER

- a. WORKING BLOTTING BUFFER should be **prepared and used within 5 days.**
- b. Dilute 1 volume of BLOTTING BUFFER (10x) with 9 volumes of reagent grade water. Mix well.
- c. Use 1.0 g of BLOTTING POWDER per 20 mL of the diluted BLOTTING BUFFER prepared in Step 2b above. Mix thoroughly to dissolve the powder. WORKING BLOTTING BUFFER may be stored at 2-8°C for 5 days.

3. WORKING CONJUGATE 1 SOLUTION

- a. Refer to the SUPPLEMENTAL INSTRUCTIONS sheet for the dilution appropriate for the CONJUGATE 1 lot supplied with the kit.
- b. WORKING CONJUGATE 1 SOLUTION should be **prepared fresh prior to use.**

4. WORKING CONJUGATE 2 SOLUTION

- a. Refer to the SUPPLEMENTAL INSTRUCTIONS sheet for the dilution appropriate for the CONJUGATE 2 lot supplied with the kit.
- b. WORKING CONJUGATE 2 SOLUTION should be **prepared fresh prior to use.**

5. WORKING SUBSTRATE SOLUTION

- a. WORKING SUBSTRATE SOLUTION should be **prepared fresh prior to use.**

Prepare WORKING SUBSTRATE SOLUTION by mixing equal volumes of SUBSTRATE A and SUBSTRATE B. **Mix well.**

Reagents Required (in mL)* for Various Number of Strips

	NUMBER OF STRIPS						
	1	3	6	9	15	20	27
DILUTED WASH BUFFER	20.0	60.0	120.0	180.0	300.0	400.0	540.0
WORKING BLOTTING BUFFER	5.0	17.0	30.0	45.0	75.0	100.0	135.0
WORKING CONJUGATE 1**	2.0	6.0	12.0	18.0	30.0	40.0	54.0
WORKING CONJUGATE 2**	2.0	6.0	12.0	18.0	30.0	40.0	54.0
SUBSTRATE A	1.0	3.0	6.0	9.0	15.0	20.0	27.0
SUBSTRATE B	1.0	3.0	6.0	9.0	15.0	20.0	27.0

* Minimum volumes. Prepare a slight excess of each solution to compensate for loss during pipetting.

** See SUPPLEMENTAL INSTRUCTIONS sheet for Dilution Calculation.

STORAGE INSTRUCTIONS

1. Store Cambridge Biotech HIV-1 Western Blot Kits and/or individual reagents at 2-8°C.
2. Unused NITROCELLULOSE STRIPS should be kept dry and in the dark, in their storage tube, at 2-8°C.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

Changes in the physical appearance of the reagents supplied may indicate instability or deterioration of these materials. SUBSTRATE A should be colorless. If SUBSTRATE A shows color, it has become oxidized and should not be used.

KNOWN INTERFERING SUBSTANCES

Sodium azide interferes with horseradish peroxidase activity. Household bleach, when present in the wells at any stage of the assay, inhibits the ability of the assay to detect specific antibodies to HIV-1 viral components. When present at the substrate addition stage, this inhibition is accompanied by a visible fading of band intensity following the initial band development. The introduction of bleach into the test by any route (e.g., aerosol, liquid, as a residual contaminant on trays) should be avoided.

ASSAY PROCEDURE

MATERIALS PROVIDED FOR URINE SPECIMEN TESTING

- NITROCELLULOSE STRIPS 27 Strips
- WASH BUFFER (20x) 1 Bottle
(60 mL minimum/bottle)
- BLOTTING BUFFER (10x) 1 Bottle
(18 mL minimum /bottle)
- CONJUGATE 1 1 vial (Blue Cap)
(160 µL minimum/vial)
- CONJUGATE 2 1 vial (Black Cap)
(160 µL minimum/vial)
- SUBSTRATE A 1 Bottle
(30 mL minimum/bottle)
- SUBSTRATE B 1 Bottle
(30 mL minimum/bottle)
- BLOTTING POWDER 1 Package
(9.0 g minimum)
- INCUBATION TRAYS 3 Trays and Tops
- NEGATIVE URINE CONTROL 1 Bottle (Translucent with a green bar)
(10 mL minimum/bottle)
- LOW POSITIVE URINE CONTROL 1 Bottle (Amber with a purple bar)
(10 mL minimum/bottle)
- HIGH POSITIVE URINE CONTROL 1 Bottle (Amber with a red bar)
(10 mL minimum/bottle)

MATERIALS REQUIRED - NOT PROVIDED

- ROCKER OR ROTARY PLATFORM
- PIPETS
- PIPETTORS AND TIPS
- ASPIRATOR WITH DISINFECTANT TRAP
- TWEEZERS OR FORCEPS

URINE SPECIMEN COLLECTION AND PREPARATION

1. Polypropylene containers are recommended for collection, shipping or storing urine specimens.
2. Urine specimens without preservative or specimens preserved with Stabilur™ (R.P. Cargille Laboratories, Inc., Cedar Grove, NJ) may be used.
3. Urine specimens may be stored at 2-8°C for up to 18 months, or at ambient temperature (23-27°C) for up to 55 days. DO NOT FREEZE. The reliability of results with frozen urine samples has not been demonstrated.
4. Mix specimens well prior to testing.
5. If specimens are shipped, they should be shipped in accordance with requirements for transporting etiological agents.

Note: Collectors should acknowledge by initialing a sticker, that they have placed the sticker on the collection cup before a specimen was collected and have provided the test subject with the Subject Information Brochure. Urine specimens which do not include a sticker with initials of the collector must not be tested for HIV antibody using the Maxim HIV-1 Urine Western blot test.

URINE ASSAY PROCEDURE

CAUTION: When handling the incubation tray supplied with the kits, take care not to splash or mix specimens. Remove the lid carefully to prevent moisture which may condense on the lid from falling into the tray. Do not handle samples or sample loaded pipet tips over uncovered incubation trays. Splashing or aerosols may lead to cross-contamination of sample wells.

WHENEVER URINE SPECIMENS ARE ASSAYED WITH THE CAMBRIDGE BIOTECH HIV-1 URINE WESTERN BLOT KIT, THE NEGATIVE, LOW POSITIVE, AND HIGH POSITIVE URINE CONTROLS MUST BE ASSAYED.

1. Bring all reagents to room temperature prior to use (approximately 30 minutes).
2. Add 2.0 mL of DILUTED WASH BUFFER to each well to be used.

3. Using forceps, carefully remove a NITROCELLULOSE STRIP from the vial in sequential order beginning with the number 1 and place numbered side up into a well containing DILUTED WASH BUFFER.
4. Place the tray on a rocker or rotary platform for 5 to 10 minutes at room temperature, then remove the buffer by aspiration.
5. Add 1.0 mL of WORKING BLOTTING BUFFER to each well which is to contain a urine control or urine specimen.
6. Add 1.0 mL of each undiluted urine control or urine specimen to a well containing its assigned strip in WORKING BLOTTING BUFFER. CAUTION: Use a different pipet tip for each sample.
7. Cover the tray and incubate on a rocker or rotary platform overnight (14-20 hours) at room temperature (20 to 28°C).
8. Carefully uncover the tray to avoid splashing or mixing of specimens. Remove condensation or droplets on the incubation tray lid by rinsing with DILUTED WASH BUFFER or wiping with absorbent towels.
9. Aspirate the mixture from the wells into a trap containing 5% sodium hypochlorite household bleach as a disinfectant. Rinse aspirator tip with DILUTED WASH BUFFER, or reagent grade water between samples to avoid cross-contamination. Discard waste in the trap prior to substrate preparation.
10. Add 2.0 mL of DILUTED WASH BUFFER to each strip and incubate on a rocker or rotary platform for a minimum of 5 minutes. Aspirate the wash buffer. Repeat two additional times.
11. Add 2.0 mL of WORKING CONJUGATE 1 SOLUTION (prepared as directed in the SUPPLEMENTAL INSTRUCTIONS sheet) to each well. Incubate for 120 minutes at room temperature on the rocker or rotary platform.
12. Aspirate the WORKING CONJUGATE 1 SOLUTION from the wells. Wash each strip three times for a minimum of 5 minutes as in Step 10, above.
13. Add 2.0 mL of WORKING CONJUGATE 2 SOLUTION (prepared as directed in SUPPLEMENTAL INSTRUCTIONS sheet) to each well. Incubate for 120 minutes at room temperature on the rocker or rotary platform.
14. Aspirate the WORKING CONJUGATE 2 SOLUTION from the wells. Wash each strip three times for a minimum of 5 minutes as in Step 10, above.
15. Add 2.0 mL of WORKING SUBSTRATE SOLUTION to each well and incubate at room temperature on the rocker or rotary platform for 10-15 minutes. The LOW POSITIVE URINE CONTROL must exhibit a gp160 band.

16. Aspirate the WORKING SUBSTRATE SOLUTION and stop the reaction by rinsing the strips three (3) times with at least 2.0 mL of reagent grade water.

NOTE: Some specimens may cause spots to form on the strip due to precipitation. A cotton swab dipped in reagent grade water can be used to carefully remove the spots and allow for better visualization of results.

Air dry the strips between absorbent paper towels and score as described in the INTERPRETATION OF URINE RESULTS section. For best results and consistency, strips should be scored soon after air drying. The strips can then be mounted and stored between clear plastic sheets. When mounting with tape, do not tape over developed bands. This will cause bands to fade.

17. If desired, the strips may be photographed using high resolution film. Developed strips will retain their color if stored in the dark. Exposure to light and air will eventually cause bands to fade.

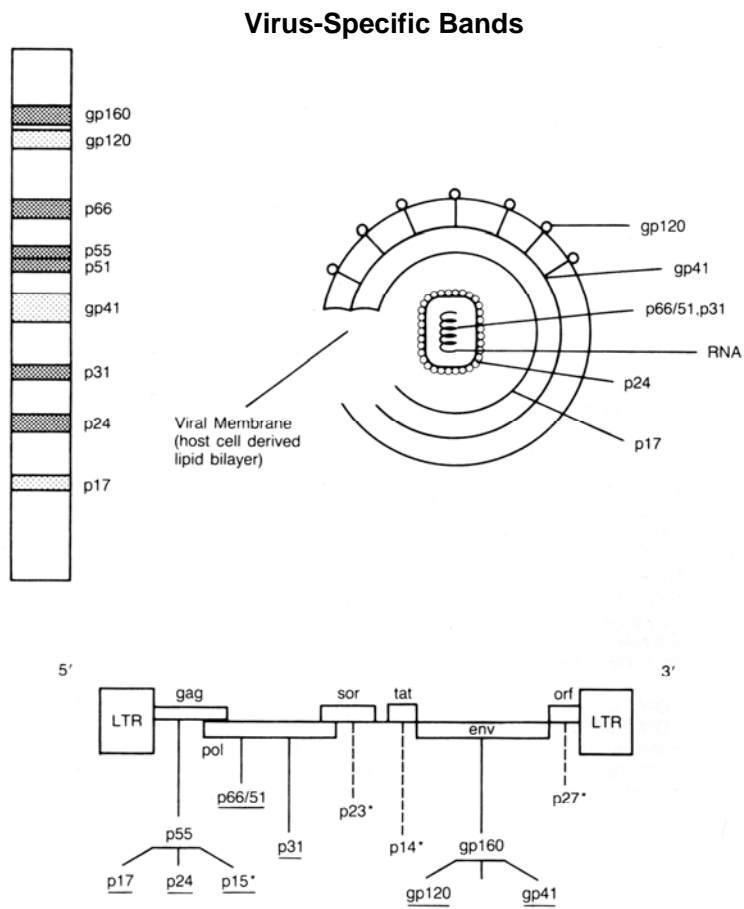
URINE QUALITY CONTROL

The NEGATIVE, LOW POSITIVE and, HIGH POSITIVE URINE CONTROLS must be included with each run of urine specimens regardless of the number of specimens tested or NITROCELLULOSE STRIPS used. The HIGH POSITIVE URINE CONTROL is used to establish the location of the gp160 band for comparison with specimen strips.

In order for the results obtained from any run of urine specimens to be considered valid, the following conditions must be met:

1. **NEGATIVE URINE CONTROL:** No bands should be visible on the NITROCELLULOSE STRIP used to test the NEGATIVE URINE CONTROL.
2. **HIGH POSITIVE URINE CONTROL:** The NITROCELLULOSE STRIP used to test the HIGH POSITIVE URINE CONTROL must exhibit a band at gp160. Additional bands may appear but are not required to demonstrate acceptable performance. Figure 1 will permit location and identification of the gp160 band observed for the HIGH POSITIVE URINE CONTROL.
3. **LOW POSITIVE URINE CONTROL:** The NITROCELLULOSE STRIP used to test the LOW POSITIVE URINE CONTROL must exhibit a band at the gp160 region, and that band must be visibly less intense than the gp160 band on the HIGH POSITIVE URINE CONTROL. Other bands may be observed, but are not required to determine acceptable performance.

Figure 1



*These HIV-1 proteins generally do not appear with Cambridge Biotech HIV-1 Urine Western Blot Kit

INTERPRETATION OF URINE RESULTS

The presence or absence of antibodies to HIV-1 in a specimen and the identity of any antibodies present are determined by comparison of each NITROCELLULOSE STRIP to the strips used for the NEGATIVE, LOW POSITIVE, AND HIGH POSITIVE URINE CONTROLS tested with that run.

The interpretation process requires three steps. First, each gp160 band which appears on a test strip must be identified based on the HIGH POSITIVE URINE CONTROL strip. Second, the gp160 band is assigned a reactivity score based on its intensity when compared to the gp160 band on the LOW POSITIVE URINE CONTROL strip. Third, the strip is interpreted based on the reactivity.

The major HIV-1 gene products that have been identified⁵⁻¹⁰ are as follows (see Figure 1).

gp160	-	Precursor of ENV glycoprotein
gp120	-	Outer ENV glycoprotein
p66	-	Reverse Transcriptase component of POL translate
p55	-	Precursor of GAG proteins
p51	-	Reverse Transcriptase component of POL translate
gp41	-	Transmembrane ENV glycoproteins
p31	-	Endonuclease component of POL translate
p24	-	GAG protein
p17	-	GAG protein

NOTE: The gp160 band may, in many cases, represent a multimer of gp41.¹¹ However, the presence of gp120 has been verified using specific mono and polyclonal antibodies. The primary response of most ENV reactive antibodies on Western Blot is to the transmembrane part whether it is a tetramer or derived from the precursor.

Figure 1 is used to locate and identify the gp160 band on the strip used with the HIGH POSITIVE URINE CONTROL. This strip is then used to identify the gp160 band location on strips used to test urine specimens.

Intensity of the gp160 band present on strips used to test specific specimens may be scored as follows:

Intensity Of Band	Reactivity Score
• Absent	-
• Less than the intensity of the gp160 band on the LOW POSITIVE URINE CONTROL strip	±
• Greater than or equal to the intensity of the gp160 band on the LOW POSITIVE URINE CONTROL strip.	+

Interpret the result of blotting as NEGATIVE, INDETERMINATE, or POSITIVE based on the pattern which is present, according to the following table:

PATTERN	INTERPRETATION
No bands present.	NEGATIVE
Any bands present but pattern does not meet the criterion for POSITIVE	INDETERMINATE
The presence of a gp160 band with a reactivity score of +. *Other bands may or may not be present	POSITIVE*

Failure to obtain the established band criteria for the NEGATIVE, LOW POSITIVE, OR HIGH POSITIVE URINE CONTROLS may be indicative of unsatisfactory test performance. Such discrepancies may be related to the following sources of variability:

- Cross-contamination of negative control reagents with high titer specimens
- Cross-contamination of reactive control reagents with non-reactive specimens
- Improper equipment operation
- User reagent preparation

INDETERMINATE results must be taken as suspect and SHOULD TRIGGER REPEAT AND FOLLOW-UP TESTING. INDETERMINATE ASSAY RESULTS MUST NOT BE CONSIDERED POSITIVE OR NEGATIVE. The correct evaluation in such situations must be based on subsequent blot testing and clinical evaluation. In such cases, INDETERMINATE blots may offer useful information.

NOTE: A person who has antibodies to HIV-1 is presumed to be infected with the virus, except that a person who has participated in an HIV vaccine study may develop antibodies to the vaccine and may or may not be infected with HIV. Clinical correlation is indicated with appropriate counseling, medical evaluation and possibly additional testing to decide whether a diagnosis of HIV infection is accurate.

REPORTING OF RESULTS

1. When reporting results, Medical Directors and ordering physicians are required to properly notify test subjects that the Maxim HIV-1 Urine EIA followed by the Cambridge HIV-1 Urine Western blot, as necessary, have decreased sensitivity compared with testing a blood sample and decreased specificity for high risk subjects compared with testing a blood sample.
2. Test results should be reported to the ordering physician or someone under the supervision of the ordering physician.
3. Test results provided to subjects should only be based on complete EIA and Western blot testing, as necessary.

LIMITATIONS OF THE URINE PROCEDURE

Optimal assay performance requires strict adherence to the assay procedure described in this insert. Deviations from the procedure may lead to aberrant results.

Slight ambiguities exist in the designation of the molecular weights of the HIV-1 antigens. The designations listed in Figure 1 have been established by both internal testing with known markers

and consensus of published literature.⁵⁻¹⁰

Although a blot POSITIVE for antibodies to HIV-1 indicates infection with the virus, a diagnosis of Acquired Immunodeficiency Syndrome or AIDS can only be made clinically if a person meets the case definition of AIDS established by the Centers for Disease Control.⁴ POSITIVE blot results using any specimen type (serum, plasma, or urine) should be followed with additional testing. Such testing may rely on alternative test methods or specimen types. The clinical implications of antibodies to HIV-1 in an asymptomatic person are not known. However, a larger proportion of such persons have virus detectable in their peripheral blood and some will develop immunodeficiency.⁹⁻¹⁰

INDETERMINATE blots should not be used as the basis for diagnosis of HIV-1 infection. However, such findings may provide useful information in the context of medical evaluation for which clinical information is available.

DUE TO VARIATIONS IN TEST PERFORMANCE AND THE UNCERTAINTY ASSOCIATED WITH INDETERMINATE BLOTS, IT IS RECOMMENDED THAT ALL INDETERMINATE BLOTS BE REPEATED USING THE ORIGINAL SPECIMEN. INDIVIDUALS WITH A BLOT THAT IS INDETERMINATE DUE TO A \pm gp160 BAND SHOULD BE RETESTED AS SOON AS POSSIBLE USING A FRESH SPECIMEN. PERSONS WITH OTHER INDETERMINATE PATTERNS SHOULD BE RETESTED USING A FRESH SPECIMEN AFTER SIX MONTHS. A NEGATIVE BLOT DOES NOT EXCLUDE THE POSSIBILITY OF INFECTION WITH HIV-1.

PERFORMANCE CHARACTERISTICS

PERFORMANCE STUDIES

Three studies were conducted to evaluate the performance of the Cambridge Biotech HIV-1 Urine Western Blot Kit using a urine assay procedure. 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 Urine 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 STUDIES

Sensitivity In HIV-1 Seropositive Individuals

The sensitivity of the Cambridge Biotech HIV-1 Urine 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 are shown in Table A.

In the combined studies, the Cambridge Biotech HIV-1 Urine 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 Urine 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 Urine 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	129	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 95.8% (69 of 72) 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	
High	RR	N 17	Pos	N 17	RR	N 90	Pose	N 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 Urine 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 Urine Western Blot Kit (N=391^a)

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

^d RR indicates repeatedly reactive in the Maxim 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 Urine 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	N	Frequency of Virus Specific Bands ^b								
			p17	p24	p31	gp41	p51	p55	p66	gp120	gp160
RR ^c N=215	Pos ^e	213	50	104	130	199	114	44	166	210	213 ^d
	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 Maxim 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 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 E 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 Urine 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
NR ^c N=533	Pos ^e	533	136	301	319	462	317	118	433	512	533 ^d
	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.
- ^c RR indicates repeatedly reactive in the Maxim HIV-1 Urine EIA.
- ^d 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).
- ^e 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 Urine 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**

N	No Intensity Criterion			Using gp160 Band Intensity Criterion Western Blot Results					
	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 STUDIES

Specificity in Low Risk Groups

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

Risk Group	N	Serum			Urine			
		Western Blot Results ^c			EIA RR	Pos ^d	Western Blot Results	
		Pos	Neg	Ind			Neg	Ind
Low^a	200^b	N T	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 Urine 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 Urine 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 Urine 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 Urine Western Blot Kit (N=515^a)

Urine EIA	Urine WB		Frequency of Virus Specific Bands ^b								
	Pos	N	p17	p24	p31	gp41	p51	p55	p66	gp120	gp160
NR ^c N=514	Pos	0	0	0	0	0	0	0	0	0	0
	Ind	0	0	0	0	0	0	0	0	0	0
RR ^d N=1 ^e	Pos ^e	0	0	0	0	0	0	0	0	0	0
	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 Maxim HIV-1 Urine EIA.
- ^d RR indicates repeatedly reactive in the Maxim 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 Urine 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 Urine Western Blot Kit (N=109^a)

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

- ^a All 109 specimens were from Study 3.
- ^b NR indicated non-reactive in the Maxim HIV-1 Urine EIA.
- ^c RR indicates repeatedly reactive in the Maxim 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 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 Urine 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 Urine 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
NR ^b N=114	Pos	0	0	0	0	0	0	0	0	0	0
	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 Maxim HIV-1 Urine EIA.

Specificity in Subjects with Medical and Other Conditions

The specificity of the Cambridge Biotech HIV-1 Urine 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.

Sensitivity in HIV-1 Seropositive Individuals
Table L

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

Group	N	Urine EIA Results		Urine Western Blot Results		
		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.

ANALYTICAL SENSITIVITY

Dilution of Paired Urine and Serum Specimens
Dilution of Matched 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 Urine 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 the Cambridge Biotech HIV-1 Western Blot Kit

No.	Specimen No.	Clinical Classification*	gp160		gp120		gp41		p24	
			Urine	Serum	Urine	Serum	Urine	Serum	Urine	Serum
1	SP0000604	AIDS	1:500	>1:50,000	1:10	>1:50,000	1:10	>1:50,000	-	-
2	SP0000606	AIDS	1:50	>1:50,000	1:2	>1:50,000	1:2	>1:50,000	-	1:1,000
3	SP0000709	HIV-1 Symptomatic	1:10	>1:50,000	-	>1:50,000	-	>1:50,000	-	1:10,000
4	SP0000752	HIV-1 Symptomatic	1:50	>1:50,000	1:2	>1:50,000	1:2	>1:50,000	1:10	>1:50,000
5	SP0000783	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

v (-) 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 Urine 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 Urine 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)**

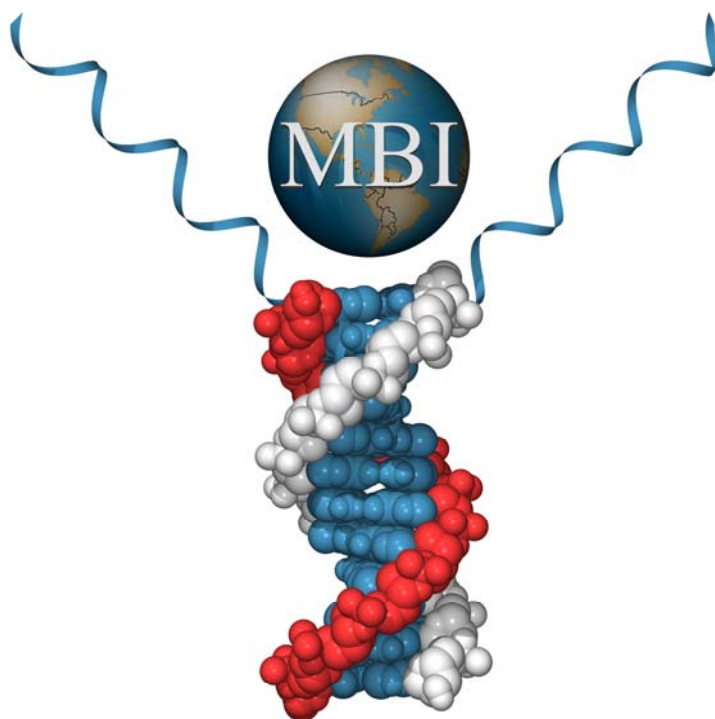
Spec/ Res ^a	# of Reps ^h	p17	p24	p31	gp41	p51	p55	p66	gp120	gp160
HPC ^b	24	23 (96)	24 (100)	24 (100)	24 (100)	15 (63)	13 (54)	24 (100)	24 (100)	24 (100)
LPC ^c	24	3 (13)	24 (100)	23 (96)	17 (71)	6 (25)	0 (0)	21 (88)	24 (100)	24 (100)
NC ^d	24	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1 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

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