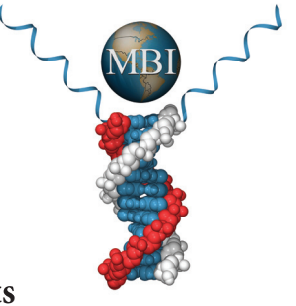
MAXIM HIV-1 URINE EIA

Enzyme Immunoassay for the Detection of Antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) in Urine



Cat. No. 700001, 192 Tests

Cat. No. 700000, 480 Tests

FOR IN VITRO DIAGNOSTIC USE

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NAME AND INTENDED USE

The Maxim 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, specimens that are repeatedly reactive using this test should be further tested only using the additional, more specific, Cambridge Biotech HIV-1 urine Western blot Kit (PN 98078).

SUMMARY AND APPLICATION

Note: See Warnings, Interpretation of Results, Limitations of the Procedure and Performance Characteristics sections for information on:

1. Reduced sensitivity and specificity of testing urine specimens compared with testing blood specimens. The Maxim HIV-1 Urine EIA gave a false negative rate of 1.3% for a combined population of AIDS patients and other HIV-1 seropositive individuals. The Maxim HIV-1 Urine EIA gave a false positive rate of 0.9% in low risk populations, 9.7% false positive rate for patients at high risk for HIV-1 infection, 14% false positive rate when testing subjects with unknown risk factors for HIV-1 and 18% false positive rate for patients with other medical conditions.
2. The requirement for laboratories using the test to provide ordering physicians with Subject Information Brochures and stickers.
3. The requirement for the test subject to sign or initial the "Subject Information Brochure" sticker and to affix the sticker to the urine collection container. Laboratory testing should be performed only on specimens which have a signed or initialed sticker on the urine collection container.
4. Reporting test results to the ordering physician or someone under the supervision of the ordering physician.
5. Results from this test are not to be used for screening potential blood donors.

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 the 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[3]. Most individuals infected by HIV develop antibodies to the major structural proteins of HIV[13]. Detection of these antibodies in blood has long been considered prerequisite for the diagnosis of AIDS[8,14]. Recent evidence also demonstrates the detection of antibodies to HIV in the urine of HIV infected individuals[4,10,11].

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[15] 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 (Cambridge Biotech HIV-1 Urine Western Blot Kit – PN 98078) 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.

PROCEDURE PRINCIPLES

The Maxim 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 absorbed onto the wells of a microwell plate. Urine specimens, controls or calibrators which may contain antibodies to the 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, control or calibrator. 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.

Samples that are initially reactive should be retested in duplicate using the originally collected specimen. If after repeat testing, one or both of the duplicate tests are reactive, the specimen is considered repeatedly reactive in the test. Before a determination of HIV-1 status can be made, subjects with repeatedly reactive results should be further evaluated using only the additional, more specific, Cambridge Biotech HIV-1 Urine Western Blot Kit (PN 98078).

Maxim HIV-1 Urine EIA, 192 or 480 Tests

Label	Component	Contents
1	**RECOMBINANT gp160 HIV-1 ANTIGEN COATED MICROWELL PLATE** One plate holds 6 – 2x8-well strips (96 wells), with adsorbed recombinant gp 160 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)
11	**PLATE SEALERS** Twenty five sealers per package.	1 Package or 2 Packages

WARNINGS

For In vitro Diagnostic Use

FDA has licensed this test for use with urine specimens only. Use of this licensed test kit with other types of specimens may result in inaccurate results.

1. Only urine specimens without preservatives or specimens preserved with StabilurTM (R.P. Cargille Laboratories, Inc. Cedar Grove, NJ) may be used in this assay.
2. Specimens preserved with StabilurTM may have lowered absorbance values and reduced sensitivity.
3. HIV-1 antibody testing of urine specimens has reduced sensitivity and specificity compared with HIV-1 antibody testing of blood specimens (see Performance Characteristics section for details).
4. Due to the possibility of false positive (i.e. EIA repeatedly reactive) HIV-1 test results, subjects with repeatedly reactive results should be further evaluated using only the additional, more specific, Cambridge Biotech HIV-1 Urine Western Blot Kit (PN 98078).

PRECAUTIONS

1. The positive control and negative calibrator are heat-treated to inactivate viruses. However, handle assay specimens, controls and calibrators as if capable of transmitting infectious agents. Use of good laboratory working practices and CDC-NIH guidelines 7 is recommended.
2. All test operators should adhere to the Occupational Safety and Health administration (OSHA) regulations (29 CFR 19.10).
3. Keep testing area separate from areas in which blood or blood products for transfusion are stored.
4. Do not use reagents beyond the expiration date printed on the reagent label.
5. With the exception of Substrate Tablets, Substrate Diluent, Wash Solution, and Stop Solution, do not interchange reagents from different lots of kits.
6. Do not interchange bottle caps.

7. Mix all liquid reagents by gently inverting 3 to 5 times, just prior to use.
8. Prior to performing the test, bring to room temperature only as many strips of micowells as are needed to perform the test run. Any strips of microwells which are not to be used in the current test run, should be sealed in the foil bag with desiccant and stored at 2-8°C.
9. Remove reagents from refrigerated storage approximately 60 minutes before beginning the assay. Bring kit reagents to room temperature (15-30°C) prior to use. Return all kit components to their recommended storage conditions immediately after use.
10. Avoid microbial contamination and cross contamination of reagents and specimens. Use separate pipets and/or pipet tips, and reagent reservoirs for each component of the kit and for each specimen to be tested.
11. Open the foil wrapping containing the Substrate Tablet by tearing at the indentation while holding the tablet within the foil. Do not touch the Substrate Tablet with fingers. Invert the foil wrapper and drop the tablet(s) into the container used for preparation of substrate solution. Substrate solution which has turned yellow in color before use should be discarded.
12. Avoid contact of Substrate Tablets with skin and mucous membranes. If this reagent comes into contact with skin or mucous membranes, flush thoroughly with water.
13. Do not reuse plate sealers.
14. Proceed immediately to add the next reagent after each wash step. Do not allow the micowells to dry out.
15. Mix specimens thoroughly by inversion before adding them to the test wells.
16. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
17. Do not pipette by mouth.
18. Avoid splashing or creating aerosols.
19. Wipe spills promptly with a 0.5% sodium hypochlorite solution (1:10 dilution of liquid household bleach). Do not place solutions containing bleach in the autoclave.
20. Dispose of all specimens and materials used in the procedure according to local regulations.
21. Some of the reagents in this kit contain sodium azide as a preservative.

Sodium azide has been reported to form lead or copper azide in plumbing. These azides are explosive. Flush drains thoroughly after disposing of solutions containing sodium azide to prevent azide build-up. Check with local regulations for disposal restrictions.

22. Wear protective clothing and disposable gloves while handling the kit regents. Wash hands thoroughly after performing the test.

STORAGE

The recommended storage condition for this kit and its components is 2-8°C. Allow approximately 60 minutes for kit reagents and components to reach room temperature (15-30°C) prior to beginning the assay. Return reagents to their labeled storage condition immediately after use.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision pipets to deliver 10 µL, 25 µL, 50 µL, 100 µL, 200 µL and 1000 µL
2. Disposable pipet tips
3. Serologic pipets, pipet bulb or equivalent
4. Timer
5. Microplate washer or hand held aspirator and a vacuum source
6. Microplate reader capable of measuring absorbance at a wavelength of 405 nm (Reference filter at 630 nm, optional)
7. Polypropylene and/or polyethylene containers for preparation of diluted buffers and reagents
8. Paper towels or absorbent paper
9. Deionized water or equivalent
10. Dry-heat incubator capable of maintaining 37°±1°C
11. Uncoated microwell strips, "Null strips"(Catalog number 110100)
12. Household bleach (5-8% hypochlorite)
13. Reagent reservoirs or troughs (optional)
14. Vortex (optional)
15. Disposable gloves

SPECIMEN COLLECTION AND PREPARATION

The HIV-1 Urine EIA is to be performed on human urine.

Note:

Urine specimens submitted for HIV-1 testing should have the Subject Information Brochure sticker which is initialed by the test subject, and reads "I have read and understand the Subject Information Brochure for the Maxim HIV-1 EIA test" attached to the collection container. Urine specimens which do not include the initialed sticker must not be tested for HIV using the Maxim HIV-1 Urine EIA.

1. It is recommended that specimens be collected according to the NCCLS tentative guidelines (GP16-A).
2. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents. DO NOT ALLOW THE URINE SPECIMENS TO FREEZE DURING SHIPMENT.
3. Specimens visibly contaminated with bacteria, blood, or sediment may give inaccurate test results.
4. Store the urine refrigerated at 2-8°C. DO NOT FREEZE THE URINE.
5. Test urine specimens as soon after collection as possible. Only urine specimens without preservatives or specimens preserved with StabilurTM (R.P. Cargille Laboratories, Inc., Cedar Grove, NJ) may be used in this assay. Unpreserved or StabilurTM preserved urine specimens may be stored at room temperature (15-30°C) up to 55 days, or for up to one year at 2-8°C.
6. Polyethylene or polypropylene containers are recommended for shipping or storing urine specimens.

1. Microwell Plate
Remove coated microwell plate from its foil bag. Remove any unneeded strips from the plate frame, reseal them in the foil bag along with the desiccant, and return the foil bag to 2-8°C. If using a 96-well plate washer, the plate frame should be completely filled with microwells by adding as many Null strips as necessary. The 2x8-well strips of microwells can be split lengthwise into separate 1x8-well strips.

2. 1X Wash Solution
The 1X Wash Solution is a 1:10 dilution of the 10X Wash Solution provided with the kit (see HIV-1 Urine EIA Procedure Step 3). Mix the 10 X Wash Solution thoroughly by gently inverting 3 to 5 times. Prepare 1X Wash Solution by adding one part 10 X to nine parts deionized water or equivalent (e.g., 450 ml of 10X Wash Solution added to 4050 mL of Deionized Water). Mix the 1X Wash Solution thoroughly.

Diluted Wash Solution (1X) may be used for 30 days when stored at room temperature (15-30°C). Record lot number, date of preparation and expiration date on the container.

3. Conjugate Solution
Conjugate Solution must be freshly prepared each time the assay is performed by diluting Conjugate Concentrate with Conjugate Diluent (see HIV-1 Urine EIA Procedure Step 9). In order to test a complete, 96-well plate, dilute 50 µL of Conjugate Concentrate into 11 mL of Conjugate Diluent. For partial plates, use the following table. Mix the prepared conjugate solution thoroughly by gently inverting 3 to 5 times.

Preparation of Conjugate Solution

No. of coated 1x8-well strips to be used	1	2	3	4	5	6	7	8	9	10	11	12
Volume of Conjugate Conjugate (µL)	10	10	20	20	20	30	30	40	40	40	50	50
Volume of Conjugate Diluent (mL)	2.2	2.2	4.4	4.4	4.4	6.6	6.6	8.8	8.8	8.8	11	11

4. Substrate Solution

Substrate Solution must be freshly prepared each time the assay is performed by dissolving substrate Tablets in Substrate Diluent (see HIV-1 Urine EIA Procedure Step 14). In order to test a complete, 96-well plate, dissolve three Substrate tablets into 15 mL of Substrate Diluent. For partial plates, use the following table. Mix the prepared substrate solution thoroughly before use. Vortex if necessary. There should be no visible undissolved material.

Preparation of Substrate Solution

No. of coated 1x8-well strips to be used	1	2	3	4	5	6	7	8	9	10	11	12
Number of Substrate Tablets	1	1	1	1	1	2	2	2	2	2	2	3
Volume of Substrate Diluent (mL)	5	5	5	5	5	10	10	10	10	10	10	15

CAUTION: Proper reagent preparation is critical. Refer to Reagent Preparation section as appropriate throughout the procedure. Once the test has been started, each step must be performed without delay according to the package insert.

1. Allow approximately 60 minutes for kit reagents to reach room temperature (15-30°C) prior to use. Return reagents to their labeled storage condition immediately after use.
2. Prepare the 96-well plate for use according to Reagent Preparation (Step 1).
3. Prepare 1X Wash Solution according to Reagent Preparation (Step 2).
4. Identify well position(s) for each specimen on a data sheet, plate map, or by an automated bar-code identification system. Each plate, or partial plate, of test specimens must include 2 positive control wells and 3 negative calibrator wells
5. Gently mix the bottle of Sample Buffer by inverting 3 to 5 times. Only remove as much Sample Buffer as required for immediate testing purposes. Add 25µl of Sample Buffer to each coated microwell that will contain a specimen, calibrator or control. Do not return unused portion to the bottle.
6. Add 200 µL of each specimen, calibrator or control to the bottom of each microwell according to the positions identified in Step 4. All microwells containing calibrators, controls and test specimens must be subjected to the same process and incubation conditions.

CAUTION: Use a separate pipet tip for each specimen, positive control, or negative calibrator, to minimize the potential for cross-contamination.

7. Cover each plate securely with a plate sealer and ensure that the edges are completely sealed.
8. Incubate the plates at 37°±1°C for 60 minutes ±5 minutes.
9. Prepare conjugate solution according to Reagent Preparation (Step 3).

10. Washing Procedure:
    a. At the end of the incubation, carefully remove the plate sealer, avoiding splashing, and discard it in an appropriate waste receptacle.
    b. Completely aspirate the liquid from the microwells by using a plate washer or a hand held aspirator connected to a vacuum source.
    c. Fill the microwells with 1XWash Solution (approx. 350 µL) and immediately aspirate.
    d. Repeat Step 10c an additional 5 times for a total of 6 washes.
    e. Grasp the microwell plate firmly by the long sides in order to secure the strips firmly in the plate frame. Blot the plate by inverting the plate and vigorously tapping it on a clean absorbent towel.
11. Dispense 100 µL of conjugate solution into each coated microwell which received a specimen, calibrator or control.
12. Cover each plate with a plate sealer and ensure that the plate is well sealed along the edges.
13. Incubate at 37°±1°C for 60 minutes ±2 minutes.
14. Prepare substrate solution according to Reagent Preparation (Step 4) as soon as the conjugate incubation has started. Ensure that the tablets have completely dissolved before using the solution.
15. Perform a wash procedure as in Step 10.
16. Dispense 100µL of substrate solution into each microwell which received a specimen, calibrator or control. Do not use the substrate solution if it has developed a yellow color or if the tablets have not completely dissolved.
17. Cover each plate securely with a plate sealer and ensure that the plate is well sealed along the edges.
18. Incubate the plates at 37°±1°C for 30 minutes ±2 minutes.
19. At the end of the incubation, carefully remove the plate sealer, avoiding splashing, and discard it in an appropriate waste receptacle.
20. Add 50 µL of Stop Solution to each microwell which received a specimen, calibrator or control.

21. Blank the Microplate reader on air and read the plate at 405 nm. If using a dual filter instrument, the recommended reference wavelength is 630 nm. Plates should be read within 30 minutes of adding the Stop Solution.
22. Read and record the absorbance of all wells.

CALCULATION OF RESULTS

Do not use the absorbance values shown in the example as a substitute for assay controls or calibrators.

The presence or absence of antibodies to HIV-1 is determined by comparing the absorbance value of the specimen to the cutoff value. The cutoff value is determined by adding 0.180 to the mean absorbance value of the three Negative Calibrators.

Negative Calibrator Values

1. The absorbance value of each Negative Calibrator well should be less than or equal to 0.200. One Negative Calibrator value may be discarded if outside this range and the mean calculated by adding the two remaining absorbance values and dividing by 2. If two or more Negative Calibrator values are greater than 0.200, the assay is invalid and must be repeated.

2. Determine the mean of the Negative Calibrator absorbance values by adding the three absorbance values together and dividing by three.

Example;

Negative Calibrator #	Absorbance
1	0.113
2	0.091
3	0.087
	0.291

$$\text{Mean Negative Calibrator absorbance} = \frac{0.291}{3} = 0.097$$

Positive Control Values

Each Positive Control must have an absorbance value in the range of 1.200 to 2.700. No positive Control value may be discarded. If any Positive Control value is less than 1.200 or greater than 2.700, the assay is invalid and must be repeated.

Cutoff Value

The cutoff value is 0.180 plus the mean absorbance of the Negative Calibrator.

Example: 0.180 + 0.097 = 0.277

INTERPRETATION OF RESULTS

Note:

1. There is reduced sensitivity and specificity with testing urine specimens compared with testing blood specimens.

2. Test results should be reported to the ordering physician or someone under the supervision of the ordering physician.

1. Specimens with absorbance values less than the cutoff value are considered non-reactive by the criteria of the Maxim HIV-1 Urine EIA and may be considered negative for antibodies to HIV-1. Further testing is not required.

2. Specimens with absorbance values equal to or greater than the cutoff value are considered reactive (initially reactive) by the criteria of the Maxim HIV-1 Urine EIA, but before interpretation, the original specimen should be retested in duplicate.

3. Initially reactive specimens which do not react in either of the duplicate repeat tests are considered negative for antibodies to HIV-1 and further testing is no required.

4. If either duplicate retest is reactive, the specimen is considered repeatedly reactive.

5. Prior to interpretation, supplemental testing must be performed on repeatedly reactive specimens using only the Cambridge Biotech HIV-1 Urine Western Blot Kit (PN 98078). Refer to the Cambridge Biotech HIV-1 Urine Western Blot Kit (PN 98078) package insert for the testing requirements for urine specimens.

LIMITATIONS OF THE PROCEDURE

Note:

1. When reporting results, Medical directors and ordering physicians are required to properly notify test subjects that the Maxim HIV-1 Urine EIA has decreased sensitivity and specificity compared to a test on blood. Samples which are repeatedly reactive on the Maxim HIV-1 Urine EIA must be tested using only the Cambridge Biotech HIV-1 Urine Western Blot Kit (PN 98078).

2. Specimens submitted for HIV-1 testing using urine should have the Subject Information Brochure sticker attached to the collection container which reads "I have read and understand the Subject Information Brochure for the Maxim HIV-1 EIA test" and is initialed by the test subject. Specimens which do not include the initialed sticker cannot be tested for HIV using the Maxim HIV-1 Urine EIA.

1. False negative results occur more frequently when testing urine specimens compared with testing blood specimens. See Performance Characteristics sections for details. False negative results (the subject is infected, but the urine specimen test is negative) may be result of antibody levels in urine which are below the sensitivity (lower limit of detection) of this procedure. This may occur, for example, during the early phase of infection, or when there is disease condition in which urine cannot be concentrated by the kidney.

2. False positive results (i.e. the subject is not infected but the urine specimen test is EIA repeatedly reactive) occur more frequently when testing urine specimens compared with testing blood specimens. See Performance Characteristics sections for details. Supplemental testing of repeatedly reactive urine specimens should be performed using only the Cambridge Biotech HIV-1 Urine Western Blot Kit before the HIV-1 status of an individual can be determined.

3. The Maxim HIV-1 Urine EIA procedure and the Interpretation of Results must be followed closely when testing urine for the presence of antibodies to HIV-1 from individual subjects. Data regarding the interpretation were derived from testing individual urine specimens. Insufficient data are available to interpret tests performed on other body specimens, or pooled urine. Testing of these specimens is not recommended.

4. Studies to determine the performance characteristics of the Maxim HIV-1 Urine EIA in subjects younger than 18 years of age have not been performed.

5. The Maxim HIV-1 Urine EIA detects antibodies to HIV-1 in urine and thus may be useful as a test in hospitals, reference laboratories, including those carrying out public health and risk assessment, medical clinics, or other health care settings where testing serum or plasma is impractical or unavailable. Clinical studies continue to refine the interpretation and the medical significance of the presence of antibodies to HIV-1 in urine[1,2,4].

6. Serologic testing alone cannot be used to diagnose AIDS, even if the recommended investigation of repeatedly reactive specimens suggests a high probability that antibody to HIV-1 is present in a specimen. AIDS and AIDS-related conditions are clinical syndromes and their condition can only be established clinically[5,6,9].

7. A negative test result at any point in the investigation of individual subjects does not preclude the possibility of exposure to, or infection with HIV-1.

8. The risk of an asymptomatic person developing AIDS or an AIDS-related condition on the basis of repeatedly reactive test is not known.

PERFORMANCE CHARACTERISTICS

Reproducibility

Intra-assay reproducibility was evaluated for 4 lots of product by assaying at least 16 replicates of three specimens plus the positive controls and negative calibrators using one plate for each lot (n >64). Representative data for one of these four lots are shown in Table 1. Inter-assay reproducibility was evaluated using 4 product lots by repeating the intra-assay reproducibility study on three separate days (n=192). The combined data are also shown in Table 1.

TABLE 1. REPRODUCIBILITY

	INTRA-ASSAY[c]				INTER-ASSAY			
Specimen	Mean S/CO[a]	Average OD	S.D.	%CV	Mean S/CO[a]	Average OD	S.D.	%CV
1	8.218	2.375	0.205	8.6	6.661	2.021	0.354	17.5
2	2.996	0.866	0.087	10.0	2.341	0.711	0.121	17.0
3	0.363	0.105	0.014	13.3	0.401	0.123	0.023	18.7
Positive Control	6.747	1.950	0.220	11.3	6.332	1.978	0.374	18.9
Negative Calibrator	N/A[b]	0.109	0.019	17.4	N/A[b]	0.127	0.026	20.5

a. S/CO = signal to cutoff ratio
b. N/A = not applicable
c. Data for a single lot

Sensitivity

Sensitivity in HIV-1 Seropositive Individuals

The sensitivity of the Maxim HIV-1 Urine EIA was evaluated by assaying 300 paired urine and serum specimens obtained form subjects with a clinical diagnosis of AIDS[9] at eight collection sites. When the urine EIA results were compared to the serum EIA results, 297 of the 300 urine specimens were repeatedly reactive. The data are shown in Table 2. In this population the sensitivity of the HIV-1 Urine EIA was 99.0% (297/300).

TABLE 2. SENSITIVITY IN AIDS PATIENTS AND OTHER SEROPOSITIVE INDIVIDUALS
(N=1,111)

		Urine EIA Results	
Group	Number of Specimens	RR	NR
AIDS	300	297 (99.0%)	3 (1.0%)
HIV-1 Positive Symptomatic	275	271 (98.5%)	4 (1.5%)
HIV-1 Positive Asymptomatic	339	332 (97.9%)	7 (2.1%)
HIV-1 Positive Unclassified[a]	197	197 (100%)	0 (0.0%)
Total	1,111	97 (98.7%)	14 (1.3%)

RR - Repeatedly Reactive, NR - Non Reactive

a The clinical status of these HIV-1 positive subjects was unknown.

In addition to the AIDS patients tested, 275 paired urine and serum specimens from symptomatic subjects, 339 paired urine and serum specimens form asymptomatic subjects and 197 paired urine and serum specimens from subjects whose clinical status was unknown were collected from seven sites and assayed. All subjects were known to be HIV-1 antibody positive.

The results are shown in Table 2. The Maxim HIV-1 Urine EIA identified as repeatedly reactive 271 of the 275 (98.5%) specimens from symptomatic individuals, 332 of the 339 (97.9%) specimens from asymptomatic individuals, and 197 of the 197 (100%) specimens from individuals of unknown clinical status. In the combined population of AIDS patients and other HIV-1 positive subjects tested in this study, the sensitivity of the Maxim HIV-1 Urine EIA was 98.7% (1,097/1,111).

Sensitivity in High Risk Populations

In a prospective study, two hundred twenty nine (229) paired urine and serum specimens were collected from individuals considered to have one or more risk factors for HIV infection. As shown in Table 3, 107 of theses urine specimens were repeatedly reactive in the HIV-1 Urine EIA. Ninety four (94) of the repeatedly reactive urine specimens were matched to serum specimens repeatedly reactive in a licensed serum EIA and confirmed positive by Western blot. Thirteen (13) of the repeatedly reactive urine specimens were matched to serum EIA non-reactive specimens (urine EIA false positive). One urine EIA non-reactive specimen was matched to serum EIA repeatedly reactive and Western blot positive specimen (urine EIA false negative).

TABLE 3. DETECTION OF ANTIBODIES TO HIV-1 IN URINE FROM PATIENTS AT HIGH RISK FOR HIV-1 INFECTION (N=229)

		Urine EIA Results		Serum EIA Results		Serum Western Blot Results[g]		
Risk Factor	Number Tested	RR	NR	RR	NR	Pos	Neg	Ind
IV Drug Users[a]	167	93[b]	74	84	83	84	0	0
Hemophiliacs	10	8[c]	2	8	2	8	0	0
Sexual Partners of HIV-1 Infected Individuals	41	4[d]	37[f]	3	38	3	0	0
Prostitutes	11	2[e]	9	0	11	0	0	0
Total	229	107	122	95	134	95	0	0

RR - Repeatedly Reactive, NR - Non Reactive, Pos - Positive, Neg - Negative, Ind - Indeterminate

a. 163 of the subjects in this risk category had multiple risk factors.
b. Eighty four of the 93 urine specimens were matched to EIA repeatedly reactive Western blot positive serum specimens. Of the remaining specimens, 9 were matched to EIA non-reactive serum (urine EIA false positive).
c. The eight urine specimens were matched to EIA repeatedly reactive Western blot positive serum specimens.
d. Two urine specimens were matched to EIA repeatedly reactive Western blot positive serum specimens, 2 were matched to EIA non-reactive serum specimens (urine EIA false positive).
e. The 2 urine specimens were matched to EIA non-reactive serum specimens (urine EIA false positive).
f. One urine EIA non-reactive serum specimens was matched to an EIA repeatedly reactive and Western blot positive serum (urine EIA false negative).
g. Only EIA repeatedly reactive serum specimens were tested by Western blot.

In this population of subjects at risk for HIV-1 infection, the Maxim HIV-1 Urine EIA identified 94 out of 95 (99.0%) EIA repeatedly reactive Western Blot positive serum specimens.

Specificity

Specificity in High Risk Populations

In the prospective study of high risk individuals described in the previous section, Table 3 shows that there were 13 (9.7%) of the repeatedly reactive urine specimens matched to non-reactive serum specimens (urine EIA false positive). This data show that, when assayed for the presence of antibodies to HIV-1, urine specimens from subjects at high risk for HIV-1 infection may have an increased false positive rate compared with blood.

Specificity in Low Risk Populations

The specificity of Maxim HIV-1 Urine EIA was tested at two sites which routinely perform HIV antibody determinations on specimens collected from life insurance applicants throughout the United States. Life insurance applicants are assumed to be at low risk for HIV infection.

Seven thousand and eighty two (7,082) paired urine and serum specimens were tested. The results are provided in Table 4. One hundred and twenty (120) [1.7%] of the 7,082 specimens tested were urine initially reactive and 68 (1.0%) were urine repeatedly reactive. Of these 7 (0.1%) were also serum repeatedly reactive and serum Western Blot positive. Five (0.1%) were serum repeatedly reactive / urine non-reactive and serum Western blot negative or indeterminate.

TABLE 4. DETECTION OF ANTIBODIES TO HIV-1 IN RANDOMLY COLLECTED URINE FROM PATIENTS AT LOW RISK FOR HIV-1 INFECTION (N=7,082)

			Urine EIA Results			Serum EIA Results			Serum Western Blot Results[d]		
Trial	Site	Number Tested	IR	RR	NR	IR	RR	NR	Pos	Neg	Ind
1	1	1,802	32 (1.7%)	6[a] (0.3%)	1,796 (99.7%)	4 (0.2%)	4 (0.2%)	1,798 (99.8%)	1	1[e]	2[f]
	2	2,002	22 (1.1%)	10[b] (0.5%)	1,992 (99.5%)	10 (0.5%)	4 (0.2%)	1,998 (99.8%)	2	0	2[g]
2	1	3,278	66 (2.0%)	52[c] (1.6%)	3,226 (98.4%)	6 (0.2%)	4 (0.1%)	3,274 (99.9%)	4	0	0
Total		7,082	120 (1.7%)	68 (1.0%)	7,014 (99.0%)	20 (0.3%)	12 (0.2%)	7.070 (99.8%)	7	1	4

IR – Initially Reactive, RR - Repeatedly Reactive, NR - Non Reactive, Pos – Positive, Neg – Negative, Ind - Indeterminate

a. One of 6 urine specimens was matched to a Western blot positive serum, 5 were matched to EIA non-reactive sera (urine EIA false positive).
b. Two of 10 urine specimens were matched to Western blot positive sera, 8 were matched to EIA non-reactive sera (urine EIA false positive).
c. Four of 52 urine specimens were matched to Western blot positive sera,48 were matched to EIA non-reactive sera (urine EIA false positive).
d. Only EIA repeatedly reactive serum specimens were tested by Western Blot.
e. The matched urine specimen was EIA non-reactive.
f. The 2 matched urine specimens were EIA non-reactive (unresolved HIV status).
g. The 2 matched urine specimens were EIA non-reactive (unresolved HIV status).

In this low risk population, the Maxim HIV-1 Urine EIA identified 7 out of 7 of the EIA repeatedly reactive, Western Blot positive serum specimens. Sixty-one (61) of the 7,082 urine specimens were EIA repeatedly reactive and matched to EIA non-reactive serum specimens (urine EIA false positive). Excluding the four specimens from individuals with unresolved HIV status, the specificity of the Maxim HIV-1 Urine EIA in this study was estimated to be 99.14% (7,010/7,071), showing decreased specificity of testing urine for HIV-1 antibodies compared with testing blood.

Specificity in a Population at Unknown Risk for HIV-1 Infection

An additional 1,999 paired urine and serum specimens were collected at one site from subjects at unknown risk for HIV-1 infection (subjects visiting hospitals, clinics and doctor's offices for a variety unknown reasons and/or medical conditions). The test results are provided in Table 5 below.

TABLE 5. DETECTION OF ANTIBODIES TO HIV-1 IN RANDOMLY COLLECTED URINE FROM SUBJECTS AT UNKNOWN RISK FOR HIV-1 INFECTION (N=1,999)

	Urine EIA Results			Serum EIA Results			Serum Western Blot Results[a]		
Number Tested	IR	RR	NR	IR	RR	NR	Pos	Neg	Ind
1,999	363	299	1,700	24	23	1,976	22	0	1[b]

IR – Initially Reactive, RR - Repeatedly Reactive, NR - Non Reactive, Pos – Positive, Neg – Negative, Ind - Indeterminate

a. Only serum EIA repeatedly reactive specimens were tested by Western blot.
b. The matched urine specimen was EIA non-reactive (unresolved HIV status).

Of the 1,999 specimens tested, 363 (18.2%) urine specimens were initially reactive in the Maxim HIV-1 Urine EIA, 299 (15.0%) urine specimens were repeatedly reactive and 1,700 (85.0%) were non-reactive. Twenty-two (22) of the urine EIA repeatedly reactive specimens were matched to serum repeatedly reactive and Western blot positive specimens. In this population of subjects with unknown risk factors for HIV-1 and with unknown medical conditions, the Maxim HIV-1 Urine EIA correctly identified all 22 out of 22 serum EIA repeatedly reactive and Western blot positive specimens. Two hundred seventy seven (277) EIA repeatedly reactive urine specimens were paired to serum EIA non-reactive specimens. The HIV-1 Urine EIA false positive rate, 277/1,976 (14.0%) shows decreased specificity of testing urine for HIV-1 antibodies compared with testing blood.

Potentially Interfering Substances

Paired urine and serum specimens were collected from 375 individuals with one or more medical conditions which have potential for assay interference. The serum specimens which were matched to urine EIA repeatedly reactive specimens were tested by a licensed serum Western blot assay. The results are presented below in Table 6.

TABLE 6. DETECTION OF ANTIBODIES TO HIV-1 URINE OF PATIENTS WITH OTHER MEDICAL CONDITIONS
(N=375)

		Urine EIA Results			Serum EIA Results			Serum Western Blot Results[a]		
Patient Category	Number Tested	IR	RR	NR	IR	RR	NR	Pos	Neg	Ind
Autoimmune Conditions	49	16	10	39	1	0	49	0	0	0
Neoplasms	53	13	9	44	0	0	53	0	0	0
STD	207	50	37	170	19	4	203	2[b]	0	2[c]
Urine Conditions[d]	24	10	10	14	0	0	24	0	0	0
Multiparous	10	0	0	10	0	0	10	0	0	0
Pregnant	15	2	1	14	0	0	15	0	0	0
Exertion Dehydration	8	0	0	8	0	0	8	0	0	0
Multiple Transfusions	9	3	2	7	0	0	9	0	0	0
Total	375	94 (25.1%)	69 (18.4%)	306 (81.6%)	20 (5.3%)	4 (1.1%)	371 (98.9%)	2	0	2

IR – Initially Reactive, RR - Repeatedly Reactive, NR - Non Reactive, Pos – Positive, Neg – Negative, Ind - Indeterminate

a. Only serum EIA repeatedly reactive specimens were tested by Western blot.
b. The matched urine specimens were EIA non-reactive.
c. One matched urine specimen was EIA non-reactive, one was EIA non-reactive.
d. Includes proteinuria, bacteriuria, hematuria, and glucosuria.

Sixty-nine (69) of the urine specimens tested were repeatedly reactive in the urine EIA. Two (2) of these were also serum EIA repeatedly reactive and Western blot positive, and these specimens were correctly identified by the Maxim HIV-1 Urine EIA. One urine EIA repeatedly reactive specimen was matched to an EIA repeatedly reactive and Western blot indeterminate serum specimen. No follow-up testing was performed on this individual to determine true HIV status. The remaining 66 urine EIA repeatedly reactive specimens were matched to serum EIA non-reactive specimens. In this population of patients with other medical conditions, the HIV-1 Urine EIA false positive rate, 67/373 (18.0%) shows decreased specificity of testing urine for HIV-1 antibodies compared with testing blood.

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