

AIDS#378

**KIT AVAILABILITY**

**HIV-1 Western Blot Kit**

20 Tests

Product Number 72827

U.S. License No. 1133

Manufactured by  
**Epitope, Inc.**

Distributed by  
**Organon Teknika Corporation**  
Box 15969

Beaverton, Oregon 97008

Durham, North Carolina 27704-0969

*For technical assistance,*

*contact Organon Teknika Technical Services at 1-800-682-2666.*

**HUMAN IMMUNODEFICIENCY  
VIRUS TYPE 1 (HIV-1)**

**HIV-1 WESTERN BLOT KIT**

**An Enzyme Immunoassay for the Detection of  
Antibody to Human Immunodeficiency Virus  
Type 1 (HIV-1) in Human Serum or Plasma.**

For *in vitro* Diagnostic Use

20 Tests

Store at 2-8°C

*Western blot*

**TABLE OF CONTENTS**

ENGLISH	Section 1
FRANÇAIS	Section 2
DUTCH	Section 3
ITALIANO	Section 4
ESPAÑOL	Section 5

Français

**DISPONIBILITE DU KIT**

**VIH-1 Western Blot Kit**

20 Analyses

Numéro du produit: 72827

Numéro de License aux U.S.: 1133

Fabriqué par  
**Epitope, Inc.**  
Beaverton, OR 97008

Distribué par  
**Organon Teknika Corporation**  
Box 15969  
Durham, NC 27704-0969

France:  
Organon Teknika S.A.  
5, avenue des Prés - B.P. 26  
94267 Fresnes Cedex

Dutch

**VERKRIJGBAARHEID KIT**

**HIV-1 Western Blot Kit**

20 Tests

Productnummer 72827

Amerikaanse licentie Nr. 1133

Vervaardigd door  
**Epitope, Inc.**  
Beaverton, OR 97008

Distributie door  
**Organon Teknika Corporation**  
Box 15969  
Durham, NC 27704-0969

Italiano

**DISPONIBILITÀ DEL KIT**

**Kit per HIV-1 Western Blot**

20 Test

Prodotto No. 72827

Licenza U.S. No. 1133

Prodotto da  
**Epitope, Inc.**  
Beaverton, OR 97008

Distribuito da  
**Organon Teknika Corporation**  
Box 15969  
Durham, NC 27704-0969

Español

**DISPONIBILIDAD DEL BOTIQUÍN**

**HIV-1 Western Blot Kit**

20 Exámenes

Producto Número 72827

U.S. License No. 1133

Fabricado por  
**Epitope, Inc.**  
Beaverton, OR 97008

Distribuido por  
**Organon Teknika Corporation**  
Box 15969  
Durham, NC 27704-0969

Table of Contents	Page
NAME AND INTENDED USE .....	1
SUMMARY AND EXPLANATION OF THE TEST .....	1
CHEMICAL AND BIOLOGICAL PRINCIPLES OF THE PROCEDURE .....	2
KIT COMPONENTS SUPPLIED .....	2
EQUIPMENT REQUIRED BUT NOT SUPPLIED .....	3
KIT STORAGE AND STABILITY .....	3
CHEMICAL OR PHYSICAL INDICATIONS OF INSTABILITY .....	4
SPECIMEN COLLECTION, STORAGE AND SHIPMENT .....	4
PRELIMINARY PRECAUTIONS .....	4
PROCEDURAL NOTES AND PRECAUTIONS .....	5
HIV-1 WESTERN BLOT TEST PROCEDURE .....	6
QUALITY CONTROL .....	10
INTERPRETATION OF RESULTS .....	10
LIMITATIONS OF THE PROCEDURE .....	12
PERFORMANCE CHARACTERISTICS .....	13
INTERFERING FACTORS AND SUBSTANCES .....	16
REFERENCES .....	i
KIT AVAILABILITY .....	ii

## NAME AND INTENDED USE

The HIV-1 Western Blot Kit is an *in vitro* qualitative assay for the detection of antibodies to individual proteins of the Human Immunodeficiency Virus Type 1 (HIV-1) in human serum or plasma. It is intended for use as an additional, more specific test for HIV-1 antibodies in persons of unknown risk whose serum or plasma specimens have been found to be repeatedly reactive using screening procedures such as enzyme immunoassay (EIA).

## SUMMARY AND EXPLANATION OF THE TEST

Acquired Immunodeficiency Syndrome (AIDS) is caused by at least two etiologic agents which are designated as Human Immunodeficiency Virus Type 1 (HIV-1) and Human Immunodeficiency Virus Type 2 (HIV-2).<sup>1,2</sup> Infections with HIV-2 are found primarily in parts of West Africa.<sup>2</sup> Current data indicate that HIV infections are transmitted by sexual contact, exposure to blood (including sharing needles and syringes) or certain blood products, and perinatally by mother to infant.<sup>3,4</sup> Many screening EIAs that detect the presence of viral-specific antibody to HIV-1 have been described by investigators.<sup>5</sup> The purpose of these screening assays is to detect potentially infectious units of blood in order to prevent their use in transfusion, or in the manufacture of plasma derivatives. The same assays are used as clinical diagnostic tests.

Patients with AIDS and AIDS-related conditions exhibit a high prevalence of antibody to HIV-1. This antibody has also been found in clinically asymptomatic individuals from whom HIV-1 has been cultured.<sup>6</sup>

Clinical samples have also been described that are reactive in the screening assays but do not contain HIV-1 antibody.<sup>7</sup> Some of these samples possess antibody to certain Class II HLA histocompatibility antigens that are found in some cell lines used to produce the virus. Other persons, who have had no known exposure to HIV-1, produce reactive results in the screening test for still unknown reasons. Such nonspecific results are found commonly when screening tests are used in large populations. Since the psychosocial and medical implications of a positive antibody test may be devastating, it has been recommended that additional testing be performed on such samples to validate the presence of antibody specific to HIV-1.

The Western blot assay, as described by Tsang et al.,<sup>8</sup> is useful for elucidating the specificity of the antibody response to HIV-1. In this assay, inactivated and disrupted proteins of HIV-1 are fractionated by electrophoresis according to molecular weight using a polyacrylamide gel in the presence of sodium dodecyl sulfate (SDS). The resolved protein bands are then transferred to a nitrocellulose sheet. This nitrocellulose sheet is then cut into strips which are reacted with serum specimens. If virus-specific antibody is present, binding occurs in bands corresponding to those produced by viral antigens. These bands are visualized by using a conjugate (goat anti-human immunoglobulin labeled with horseradish peroxidase), followed by a substrate for the enzyme. The presence of HIV-1-specific immunoglobulins in serum specimens is indicated by the labeling of HIV-1-specific proteins on the nitrocellulose strips. Recognized viral antigens produce bands at gp160, gp120, p65, p55, gp41, p31, p24, and p18. (Numbers refer to apparent molecular weight in kilodaltons.)

A sample that is reactive in both the EIA screening test and the Western blot is presumed to be positive for antibody to HIV-1, indicating infection with this virus except in situations of passively acquired antibody or experimental vaccination. Antibodies to HIV-2 may also react with the protein antigens of HIV-1.<sup>9</sup> Therefore, individuals infected

with HIV-2 may have reactive tests in the HIV-1 Western blot assay. Usually, however, the cross-reactivity is incomplete resulting in an indeterminate test result (see Interpretation of Results section). Individuals with positive tests should be referred for medical evaluation. A diagnosis of AIDS can be made only if an individual meets the case definition of AIDS established by the Centers for Disease Control.<sup>10</sup>

### CHEMICAL AND BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The whole cell viral lysate used in the manufacture of the HIV-1 Western Blot Kit is manufactured by Organon Teknika Corporation (U.S. license number 956). It is HIV-1 propagated in an H-9/HTLV-IIIB, T-lymphocyte cell line. It is purified by ultra-centrifugation and inactivated by treatment with nonionic detergent and heat.

When used to manufacture the preblotted strips, inactivated and denatured proteins of the HIV-1 virus are fractionated by SDS-polyacrylamide gel electrophoresis. The resolved protein bands are electrophoretically transferred to nitrocellulose sheets. These preblotted nitrocellulose sheets are cut into strips.

HIV-1 specimens, diluted in Sample Buffer, are incubated with the preblotted nitrocellulose strips. If antibodies to specific HIV-1 proteins are present in a specimen, they bind to epitopes contained in the proteins banded on the strip. Any antibody not bound is removed by washing. The conjugate, peroxidase-labeled goat anti-human immunoglobulin, is then added to the strip and allowed to incubate. It binds to antibodies already bound to viral proteins on the strip. Excess conjugate is removed by washing. The strips are then incubated with Chromagen. The color reaction is stopped by aspiration and washing.

If antibodies to specific HIV-1 proteins (p) or glycoproteins (gp) are present in the specimen in sufficient concentration, brown bands may be visible at one or more of the following positions on the nitrocellulose strip: gp160, gp120, p65, p55, p51, gp41, p31, p24 and p18 (number refers to apparent molecular weight in kilodaltons).

### KIT COMPONENTS SUPPLIED

(20 Test Kit)

#### HIV-1 Nitrocellulose Strips

20 strips

Prenumbered nitrocellulose strips, preblotted with resolved HIV-1-specific proteins; packed in a resealable plastic bag between buffer-soaked absorbent paper; buffer contains 0.1% sodium azide as a preservative.

#### Chromagen

1 bottle; 45 ml

Phosphate buffered saline containing proprietary chromagen and hydrogen peroxide; contains 0.01% thimerosal as a preservative.

#### Powdered Milk

1 bottle; 30g

Non-fat milk solids.

#### Sample Diluent Concentrate

1 bottle; 100 ml

Phosphate buffered saline with 3.0% Tween-20; contains 0.01% thimerosal as a preservative.

#### Conjugate Concentrate

1 vial; 0.1 ml

Goat anti-human IgG (heavy and light chains) labeled with horseradish peroxidase; contains 0.01% thimerosal as a preservative.

### Negative Control

1 vial; 0.2 ml

Human serum nonreactive for antibodies to HIV-1; tested negative for HBsAg and antibodies to HCV; contains 0.1% sodium azide and 0.01% thimerosal as preservatives.

### Low Positive Control

1 vial; 0.2 ml

Human serum reactive for antibodies to HIV-1; tested negative for HBsAg and antibodies to HCV; heat-inactivated to render material noninfectious for HIV-1; contains 0.1% sodium azide and 0.01% thimerosal as preservatives.

### High Positive Control

1 vial; 0.2 ml

Human serum reactive for antibodies to HIV-1; tested negative for HBsAg and antibodies to HCV; heat-inactivated to render material noninfectious for HIV-1; contains 0.1% sodium azide and 0.01% thimerosal as preservatives.

### Reaction Trays

5 each

Eight-lane disposable trays with lids.

**Caution:** The Negative Control is prepared from human plasma or serum found to be nonreactive for HIV-1 antibodies and tested for Hepatitis B surface antigen (HBsAg) and antibodies to HCV by FDA-licensed methods. The Positive Controls are prepared from anti-HIV-1 positive human plasma or serum, which was heat-inactivated to render it noninfectious for HIV-1. However, as no procedure can offer complete assurance that infectious agents are absent, all specimens of human origin should be considered potentially infectious and handled with care.<sup>11,12</sup>

### EQUIPMENT REQUIRED BUT NOT SUPPLIED

1. Refrigerator (2-8°C)
2. 37°C water bath
3. Graduated cylinders
4. Beaker or appropriate mixing vessels
5. Balance
6. Laboratory timer
7. Magnetic stir plate and stir bar
8. Test tubes: glass, polypropylene, or polystyrene (12x75 mm)
9. Precision micropipets to deliver variable volumes from 5 to 1000 µl
10. Disposable pipet tips
11. Graduated pipets to deliver volumes to 25 ml
12. Scissors
13. Forceps for strip handling (plastic or Teflon-coated)
14. Transfer pipets
15. Rotary platform, capable of rotating at ~50 to 60 rpm (or speed to achieve proper strip motion; see page 6, step 9)
16. Aspiration system
17. Repeating pipet to deliver 2 ml volumes

### KIT STORAGE AND STABILITY

1. Store all components at 2-8°C when not in use.
2. Expiration dates printed on the kit and kit components indicate the limits of stability.
3. Stability of the components after reconstitution or dilution is as follows:
  - a. Sample Buffer  
Sample Buffer is held at room temperature while performing assay except

when using overnight sample incubation. During overnight incubation, store Sample Buffer at 2-8°C, and allow it to return to room temperature before use. Discard excess buffer at completion of assay.

- b. Sample dilutions  
Test tube sample dilutions must be applied to the strips within one hour of dilution. The dilutions are stored at room temperature during that time.
  - c. Conjugate dilution  
Conjugate dilution must be prepared during the last four-minute wash and applied immediately after the wash is complete and the Sample Buffer is aspirated. Excess Conjugate dilution must be discarded.
  - d. All other kit components are supplied ready to use.
4. Keep strip bag tightly sealed. *Do not let strips dry out.*

#### CHEMICAL OR PHYSICAL INDICATIONS OF INSTABILITY

Alterations in the physical appearance of kit materials may indicate instability or deterioration.

**Note:** Sample Diluent Concentrate may contain crystals. This will not affect assay performance if crystals are dissolved before use (see page 6, step 1.a). Chromagen may contain black particulates but their presence does not affect product performance.

#### SPECIMEN COLLECTION, STORAGE AND SHIPMENT

1. No special preparation of the patient is necessary. Serum or plasma specimens may be used. No adverse effects were observed using the anticoagulants EDTA, sodium heparin, or sodium citrate. Remove serum from the clot or plasma from the red cells as soon as possible to avoid hemolysis. If necessary, clarify specimens by centrifugation prior to testing. Heat treatment (56°C for 30 minutes) of patient samples is optional. Although results are not typically affected, strip background color may be enhanced in some specimens.
2. Specimens should be free of microbial contamination and may be stored at 2-8°C for up to two weeks. Fresh serum or plasma may be stored long-term at -20°C. Specimens repeatedly frozen or thawed or those containing particulate matter may give erroneous results.
3. Specimens to be shipped must be packaged in accordance with federal regulations governing the transport of etiologic agents.

#### PRELIMINARY PRECAUTIONS

1. Keep testing area separate from areas in which blood or blood products for transfusion are stored.
2. Do not pipet by mouth.
3. Do not smoke, eat, or drink while handling test materials.
4. Wear disposable gloves.
5. Handle all materials used in the test including samples, washing solution, reaction trays, and pipets cautiously as though they are capable of transmitting infectious agents.

6. Consult a physician immediately in the event that contaminated materials are ingested or come in contact with mucous membranes or breaks in the skin.
7. Immediately clean up any spills containing potentially infectious material with a 1:10 dilution of  $\geq 5\%$  sodium hypochlorite (bleach) and dispose of the cleaning material by an appropriate method.
8. All specimens of human origin should be considered potentially infectious and handled accordingly.<sup>11,12</sup>
9. Dispose of all specimens and material used to perform the test as if they contain infectious agents. Prior to disposal, treat as follows:

Material	Disposal Procedure
Reusable items	Autoclave for 60 minutes at 121°C.
Disposable items	Incinerate.
Liquid waste	Mix with bleach to yield a final ratio of one part $\geq 5\%$ bleach to nine parts waste (1:10). Allow the mixture to stand 30 minutes before flushing down the drain.

#### PROCEDURAL NOTES AND PRECAUTIONS

**Note:** FDA has licensed this test kit for use with serum or plasma 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 or combine any kit component, including strips, with components of another kit lot.
2. The Low Positive and Negative Controls must be assayed with **each** run. The High Positive must be assayed with the first run of every package of strips, but is optional in subsequent runs. Retain this High Positive Control strip as a reference. It will be compared to the test strips run from that package to determine band identification and placement.
3. It is important that the items used to prepare and dispense Sample Buffer be scrupulously clean (a repeating pipet is preferable for dispensing the 2 ml of Sample Buffer).
4. Prime the pipet tip when measuring samples or reagents.
5. Do not perform the test in the presence of reactive vapors (e.g., from acids, alkalis, or aldehydes), dust, or residual bleach or bleach fumes; the enzymatic activity of the conjugate may be affected or reactivity may be decreased.
6. Avoid contamination of the strips and/or the buffer-soaked absorbent paper in the resealable bag during handling (this may cause false reactivity in subsequent assays).
  - a. Prior to removing the strips from the bag, clean the work surface and forceps with isopropyl alcohol.
  - b. Change gloves prior to opening the bag.
  - c. Always use clean forceps when handling strips.
7. Do not allow strips to dry out prior to sample addition. Place strips into trays only after dilutions have been made.

8. **It is essential to avoid cross-contamination between troughs, especially prior to and during sample incubation.**
- Add sample to the trough of the reaction tray, using a transfer pipet.
  - Additional precaution may be taken by placing the strips in every other trough of a tray.
  - Avoid delivering bubbles to the liquid in the troughs.
  - As soon as the dilutions have been added to all strips in a tray, cover the tray with lid.
  - Be careful to avoid dislodging fluid from the troughs when transferring trays.
  - Liquid in the troughs should not contact tray lids.
9. While a rotation speed of 50 to 60 rpm is recommended for each rotation step, this may be adjusted to achieve proper rotation as follows. It is critical that each strip is immersed in the liquid and moves freely; however, liquid must not contact the tray lid during rotation. Monitor assay results for cross contamination.
10. Incomplete or ineffective washing will compromise the assay; it is imperative to follow the wash procedure carefully.
11. Discard used disposable reaction tray as biohazardous waste. Reuse of these trays is **not** recommended.
12. Reagents and samples are brought to room temperature (20-25°C) before beginning the assay, except for Conjugate Concentrate and Chromagen, which must both remain refrigerated (2-8°C) until just prior to use. Return all reagents to 2-8°C after use.
13. **Caution:** The Controls, as well as the buffer in the absorbent paper surrounding the strips, contain sodium azide. If discarding into sewer, flush copiously with water. This helps prevent formation of metallic azides which, when highly concentrated in metal plumbing, may be potentially explosive. Decontaminate plumbing periodically according to CDC guidelines.<sup>13</sup>

#### HIV-1 WESTERN BLOT TEST PROCEDURE

- Prepare Sample Buffer as follows:
  - Check Sample Diluent Concentrate for crystals.
    - If crystals have formed, dissolve them by warming the entire bottle and its contents in a 37°C water bath for 10 minutes or until crystals are completely dissolved.
    - Allow the material to reach room temperature before use.

- b. Determine the volume of Sample Buffer to be prepared and quantity of each constituent required from the chart below.

Total number of strips to be assayed*	ml of Sample Buffer to prepare*	deionized H <sub>2</sub> O required**	ml of Sample Diluent Conc. required**	Powdered Milk required***
3-6	150	135.0	15.0	4.5
7-9	225	202.5	22.5	6.75
10-13	325	292.5	32.5	9.75
14-16	400	360.0	40.0	12
17-20	500	450.0	50.0	15

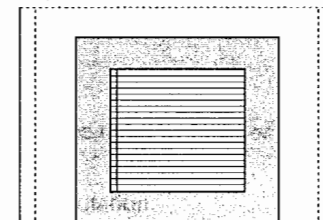
\*\*\* Include strips for controls.

\* 25.0 ml of Sample Buffer is required for each strip assayed.

\*\* Sample Diluent Concentrate is diluted 1:10 in deionized water.

\*\*\* 3 g of Powdered Milk is required for every 100 ml of diluted Sample Diluent Concentrate.

- Combine the required amounts of deionized water, Sample Diluent Concentrate and Powdered Milk.
  - Mix the solution for a minimum of 15 minutes (ensure the Powdered Milk is completely dissolved).
  - Store at room temperature while performing assay except when using overnight sample incubation. During overnight incubation, store Sample Buffer at 2-8°C, and allow it to return to room temperature before use. Discard excess buffer at completion of assay.
- Prepare an assay worksheet, ensuring that the patient sample and control identification is linked to the number embossed on the test strip.
  - Both the Low Positive and Negative Controls must be assayed with **each** run. The High Positive must be assayed with the first run of every package of strips, but is optional in subsequent runs. Retain this High Positive Control strip as a reference. It will be compared to the test strips run from that package to determine band identification and placement.
  - Prepare and add controls and patient specimens to nitrocellulose strips as follows:
    - In appropriately labeled 12x75 mm test tubes, add 40 µl of each specimen or control to 2.0 ml Sample Buffer for a dilution of 1:51. These dilutions must be tested within 1 hour.
    - Place one prenumbered strip, with *green indicator line facing up*, into each trough as follows, ensuring the strips do not dry out:
      - For initial use of strips, cut bag below the seal line, keeping upper portion of bag intact, including seal line.
      - Cut the lower portion of the bag on the two remaining sides (dotted lines as shown in diagram).
      - Fold back the packaging to expose strips.



- iv. It is recommended to begin with the left side of the series (strip #1), removing strips to be assayed and placing them in numerical order.
  - v. Grasp the strip at or above the green indicator line with forceps.
  - vi. Transfer the strips, avoiding contact with any surface, into the troughs of the reaction tray(s).
  - vii. Place any remaining strips (still encased in moist absorbent paper and contained in the lower portion of the bag) in the upper portion of bag, seal using zip closure, and return to storage at 2-8°C.
- c. Mix and carefully transfer the entire contents of each tube (~2 ml) into the corresponding trough using a transfer pipet.
5. Cover with the tray lid and mix by gentle rotation (~50 to 60 rpm) on a rotator for 10 minutes, ensuring the strips are fully immersed and moving freely in the liquid.
  6. Continue incubation using one of the following procedural options:
 

*Standard:* Incubate at room temperature with rotation (~50 to 60 rpm) for an additional 60 minutes.

*Overnight:* Incubate 16 to 24 hours at 2-8°C without rotation. (After incubation, warm Sample Buffer to room temperature before proceeding.)
  7. After incubation, completely aspirate the liquid from troughs (do not allow the strips to dry).
  8. Wash the strips immediately as follows:
    - a. Add 2.0 ml Sample Buffer to each strip.
    - b. Slide the reaction tray(s) back and forth eight (8) times by hand on a flat surface, to achieve a wave motion lengthwise in the troughs. Be sure that each strip is fully immersed in the liquid and moves freely.
    - c. Aspirate liquid completely.
    - d. Repeat steps a-c two more times.
    - e. Add 2.0 ml Sample Buffer to each strip and replace the lid(s).
    - f. Place the tray(s) on the rotator (at ~50 to 60 rpm) for four minutes.
  9. Prepare diluted Conjugate during the final wash step (step 8.f):
    - a. Conjugate dilution must be prepared during the last four-minute wash and applied immediately after the wash is complete and the Sample Buffer is aspirated. Excess Conjugate dilution must be discarded.
    - b. Remove Conjugate Concentrate from 2-8°C storage.

- c. Determine volumes required of Sample Buffer and Conjugate Concentrate from the following chart:

Total number of strips being assayed*	ml of Sample Buffer required*	µl of Conjugate Conc. required**	Total number of strips being assayed*	ml of Sample Buffer required*	µl of Conjugate Conc. required**
3	8	16	12	26	52
4	10	20	13	28	56
5	12	24	14	30	60
6	14	28	15	32	64
7	16	32	16	34	68
8	18	36	17	36	72
9	20	40	18	38	76
10	22	44	19	40	80
11	24	48	20	42	84

\* Include strips for controls.

\* Prepare 2.0 ml of diluted Conjugate for each strip assayed (a small excess has been incorporated for pipetting ease).

\*\* Conjugate Concentrate is diluted 1:501.

- d. Prepare Conjugate dilution by combining required amounts of Conjugate Concentrate and Sample Buffer, and mix well.
  - e. Return the remaining Conjugate Concentrate to storage at 2-8°C.
10. Aspirate the Sample Buffer completely and add 2.0 ml diluted Conjugate to each trough containing a strip. Replace tray lid.
  11. Incubate on rotator (~50 to 60 rpm) for 45 minutes at room temperature.
  12. After incubation, aspirate the liquid completely from troughs.
  13. Wash the strips as follows:
    - a. Add 2.0 ml Sample Buffer to each strip.
    - b. Slide the reaction tray(s) back and forth eight (8) times by hand on a flat surface, to achieve a wave motion lengthwise in the troughs. Be sure that each strip is fully immersed in the liquid and moves freely.
    - c. Aspirate liquid completely.
    - d. Repeat steps a-c three more times.
    - e. Add 2.0 ml deionized water to each trough. Replace lids.
    - f. Place the tray(s) on the rotator (at ~50 to 60 rpm) for four minutes.
  14. During final wash (step 13.f), remove Chromagen from 2-8°C storage and invert bottle two or three times to mix.
  15. Aspirate deionized water from each strip.
  16. Add 2.0 ml of Chromagen to each strip.
  17. Cover tray(s) and gently slide back and forth two to three times by hand on a flat surface.
  18. Incubate for 10 minutes at room temperature without rotation.

19. Stop the color development of the strips as follows:
  - a. Aspirate Chromagen completely.
  - b. Flood the strips with a minimum of 2.0 ml of deionized water.
  - c. Let stand for a minimum of 5 minutes.
  - d. Aspirate water completely.
20. Allow the developed strips to air dry in tray(s).
21. Handle strips carefully; use clean forceps to remove from troughs.
22. Read and interpret the **dry** strips as soon as possible since developed strips exposed to light may experience fading of bands. Store developed strips away from light at room temperature.

**QUALITY CONTROL**

Both the Negative and Low Positive Controls must be assayed regardless of the number of samples tested. A High Positive Control must be assayed with the first run of every package of strips, but is optional in subsequent runs. Retain this High Positive Control strip as a reference. It will be compared to the test strips run from that package to determine band identification and placement.

The following conditions must be met for assay results to be considered valid:

Kit Controls

1. Negative Control: No bands are observed on the strip.
2. Low Positive Control: Bands are present (P) at gp160, gp41 and p24. Other bands may or may not be visible. (*Weakly Reactive*)
3. High Positive Control: Bands are present (P) at gp160, gp41 and p24. Bands are visible at gp120, p65, p51, p31 and p18. The p55 band may or may not be visible (see Figure 1 on page 11).

**INTERPRETATION OF RESULTS**

1. Band Identification
  - a. Correlate the band position of the High Positive Control strip with Figure 1 on page 11 to identify the HIV-1 viral bands and their positions.
  - b. Compare each test strip to the High Positive Control strip for identification of reactive bands.
2. Band Intensity
  - a. Correlate the bands of each strip and control to the gp41 band on the Low Positive Control strip and assign a level of intensity as follows:

Band Intensity	Definition
Present (P)	Band intensity is greater than or equal to that of the gp41 on the Low Positive Control strip.
Indeterminate (I)	Band is visible but intensity is less than that of the gp41 on the Low Positive Control strip.
Absent (A)	No reactivity is observed.

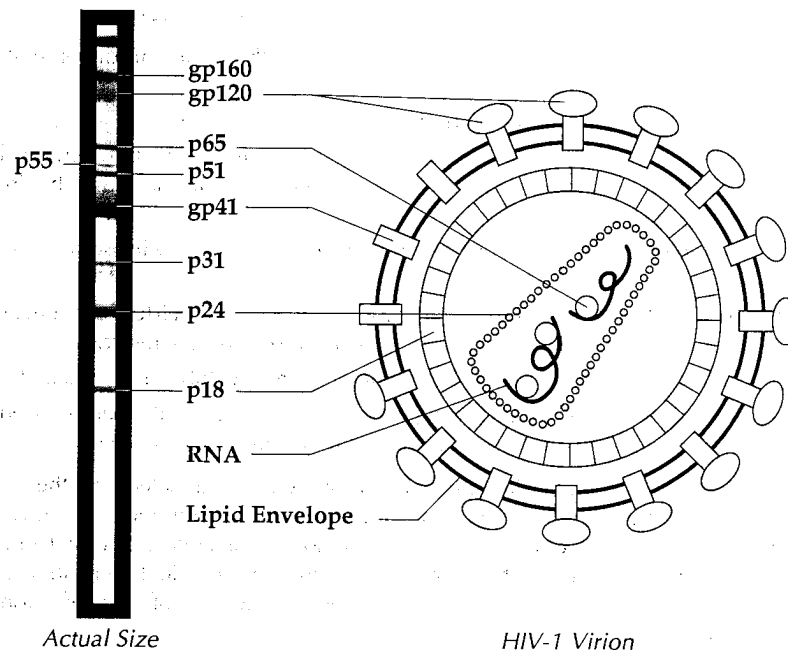
3. Strip Interpretation
  - a. Based on band position and reactivity, analyze the results and assign each strip a final result.

Test Result	Definition
Positive	Any two of the three major bands of diagnostic significance must be <i>Present</i> (as defined above). (1) gp160 and/or gp120 (2) gp41 (3) p24 Other bands may or may not be visible.
Indeterminate	Any band reactivity which does not meet the criteria for a Positive result as described above.
Negative	Band reactivity is <i>Absent</i> .

**Figure 1: Protein Band Identification on an HIV-1 Western Blot Strip**

On the left is a representation of a Western blot strip developed with High Positive Control. The illustration is a reference for band identification and position (see Interpretation of Results, page 10, step 1).

On the right is a representation of the HIV-1 virus. The bands correlate to corresponding viral subpart origin.



## Viral Origin of HIV-1 Associated Bands

Virus Gene	Gene Product and Description	
<i>env</i>	gp160	env protein precursor
	gp120	outer env protein
	gp41	transmembrane protein
<i>pol</i>	p65	reverse transcriptase
	p51	reverse transcriptase
	p31	endonuclease
<i>gag</i>	p55	core protein precursor
	p24	core
	p18	core

## LIMITATIONS OF THE PROCEDURE

- The assay must be performed in strict accordance with these instructions to obtain accurate, reproducible results.
- Although a Positive result may indicate infection with the HIV-1 virus, a diagnosis of Acquired Immunodeficiency Syndrome (AIDS) can be made only if an individual meets the case definition of AIDS established by the Centers for Disease Control.<sup>10</sup> A repeat test on an independent sample should be considered to control for sample mix-up or operator error, and to verify a positive test result.
- Individuals may present incomplete patterns due to the natural history of AIDS or other immunodeficiency states, e.g.:
  - AIDS patients may lose antibody reactions to p24 & p31;
  - Infants born to HIV-1 infected mothers, but who are uninfected, may display incomplete patterns as passively acquired maternal antibodies begin to disappear;
  - Individuals who have recently seroconverted may display incomplete band patterns;
  - Infected patients with malignancies and individuals receiving immunosuppressive drugs may fail to develop a Positive result;
  - Individuals infected with HTLV-I/II or HIV-2 may exhibit cross-reactivity;
  - Individuals may develop incomplete patterns that reflect the composition of experimental HIV sub-unit vaccines that they may have received.
- 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.
- Since reactivity of any degree with any of the virus-specific proteins present on the strip results in an Indeterminate result, all samples interpreted as Indeterminate should be repeated using the original specimen. In addition, individuals with Indeterminate results should be followed for up to six months.<sup>14</sup>
- Do not use this kit as the sole basis of diagnosis of HIV-1 infection.

- A Negative result does not exclude the possibility of HIV-1 infection. Antibody testing should not be used in lieu of donor self-exclusion by blood collection establishments.
- The HIV-1 Western Blot Kit is a biological product which, although highly consistent, does display variation from blot to blot. Examples of these variations include bands which have slightly wavy or slanted appearance, small artifacts within the banding area, and a light smearing pattern across a set of strips. These are considered normal assay variations which infrequently affect assay interpretation. However, if they do interfere with assay interpretation, call the assay invalid and repeat.

## PERFORMANCE CHARACTERISTICS

## Expected Values

The performance of this HIV-1 Western Blot Kit was evaluated in clinical studies in low risk, high risk, and AIDS populations. Sera were tested with licensed EIA test kits, a previously licensed Western blot kit, and with this kit. A total of 1,026 samples were tested by five independent clinical sites located in both high and low incidence areas. Additional populations obtained for special studies include those described under specific headings.

Specimens from low risk groups including 472 normal blood donors and 23 others of no known risk were collected both prospectively and retrospectively. Of the 345 repository samples reflecting high risk, 29 were from hemophiliacs and 102 were from the cord blood of newborns with mothers at known risk. A study was performed using 51 EIA repeatedly reactive (RR) samples from various geographic locations with high incidence of HIV-1 infection. These were comprised of 12 from Uganda, 12 from Brazil, 14 from Zambia and 13 from the USA. The remaining 163 of the High Risk population includes homosexuals, intravenous drug users (IDUs), prostitutes, sexual partners of persons at risk and those of other acknowledged risk factors. Sera were tested from 186 persons with clinically diagnosed AIDS.<sup>10</sup>

The frequency of virus-specific bands and interpretation by clinical group according to this HIV-1 Western Blot Kit is presented in Table 1.

**Table 1: Frequency of Virus-Specific Bands and Interpretation of Samples Tested by the HIV-1 Western Blot Kit**

Low Risk <sup>a</sup>	HIV-1 WB Result	Band Specificities (# and % of samples) <sup>d</sup>									Non-Viral	
		gp160	gp120	p65	p55	p51	gp41	p31	p24	p18		
EIA Neg n=254	POS	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	IND	41 (16.1)	0 (0.0)	1 (0.4)	2 (0.8)	0 (0.0)	1 (0.4)	10 (3.9)	0 (0.0)	8 (3.1)	2 (0.8)	25 (9.8)
EIA RR <sup>e</sup> n=241	POS	22 (9.1)	22 (9.9)	22 (9.1)	21 (8.7)	18 (7.5)	21 (8.7)	21 (8.7)	21 (8.7)	21 (8.7)	17 (7.0)	0 (0.0)
	IND	128 (53.1)	1 (0.4)	0 (0.0)	6 (2.5)	18 (7.5)	16 (6.6)	7 (2.9)	2 (0.8)	31 (12.9)	76 (31.5)	39 (16.2)

High Risk <sup>b</sup>	HIV-1 WB Result	Band Specificities (# and % of samples) <sup>d</sup>									Non-Viral	
		gp160	gp120	p65	p55	p51	gp41	p31	p24	p18		
EIA Neg n=195	POS	1 (0.5)	1 (0.5)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)
	IND	41 (21.0)	4 (2.1)	2 (1.0)	4 (2.1)	2 (1.0)	3 (1.5)	14 (7.2)	2 (1.0)	2 (1.0)	1 (0.5)	33 (16.9)
EIA RR <sup>e</sup> n=150	POS	140 (93.3)	140 (93.3)	140 (93.3)	136 (90.7)	107 (71.3)	137 (91.3)	138 (92.0)	133 (88.7)	134 (89.3)	115 (76.7)	0 (0.0)
	IND	3 (2.0)	2 (1.3)	0 (0.0)	2 (1.3)	18 (7.5)	1 (0.7)	1 (0.7)	0 (0.0)	2 (1.3)	76 (31.5)	1 (0.7)

AIDS <sup>c</sup>	HIV-1 WB Result	Band Specificities (# and % of samples) <sup>d</sup>									Non-Viral	
		gp160	gp120	p65	p55	p51	gp41	p31	p24	p18		
EIA Neg n=2	POS	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	IND	2 (100.0)	1 (50.0)	0 (0.4)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)
EIA RR <sup>e</sup> n=184	POS	178 (96.7)	178 (96.7)	178 (96.7)	167 (90.8)	80 (43.5)	165 (89.7)	178 (96.7)	156 (84.8)	161 (87.5)	95 (51.6)	0 (0.0)
	IND	6 (3.3)	6 (3.3)	5 (2.7)	1 (0.5)	0 (0.0)	1 (0.5)	2 (1.1)	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)

- a. Persons with no known risk factors including normal blood donors.
- b. Homosexuals, IDUs, and other accepted risk designations.
- c. CDC Classification; MMWR 1982; 31: 507-508.
- d. Band patterns for negative samples do not appear in this table. By definition, negative samples show no reactivity.
- e. RR indicates repeatedly reactive EIA results.

Table 2 summarizes Western blot results relative to the appearance of AIDS associated symptoms within EIA repeatedly reactive individuals. Since all samples were obtained from repositories, clinical symptoms were unknown in some cases.

**Table 2: HIV-1 Western Blot Results of EIA Repeatedly Reactive Samples Relative to Clinical Status**

Low Risk	Clinical Status	Western Blot (# and %)			
		EIA RR	Neg	Ind	Pos
Low Risk	Asymptomatic	3	0	0	3 (100.0)
	Unknown	238	91 (38.2)	128 (53.8)	19 (8.0)
	Total	241	91 (37.8)	128 (53.1)	22 (9.1)
High Risk <sup>1</sup>	Asymptomatic	25	0	1 (4.0)	24 (96.0)
	Symptomatic	186	0	6 (3.2)	180 (96.8)
	Unknown	123	6 (4.9)	0	117 (95.1)
	Total	334	6 (1.8)	7 (2.1)	321 (96.1)

<sup>1</sup> Includes clinically diagnosed AIDS.

**Sensitivity and Specificity**

*Gold Standard??*

Sensitivity and specificity of the HIV-1 Western Blot Kit was determined in comparative studies with a previously licensed HIV-1 Western blot using EIA repeatedly reactive samples from high AIDS risk and low risk populations respectively. The data are summarized in Table 3.

Among 256 EIA repeatedly reactive samples from high risk groups, the HIV-1 Western Blot Kit was positive in 204/204 (100%) samples identified as positive by the previously licensed test, demonstrating equivalent sensitivity.

Among 210 EIA repeatedly reactive samples from low risk groups, the HIV-1 Western Blot Kit was negative in 38/52 (73%) of samples identified as negative by the previously licensed test and indeterminate (not falsely positive) in the remaining 14 samples. Thus, there were 0% false positive tests. These data demonstrate comparable specificity of this test.

**Table 3: Comparative Study of Western Blot Results in High and Low Risk Populations**

WB Result	High Risk EIA RR				Low Risk EIA RR			
	HIV-1 Western Blot Kit			Licensed Kit Total	HIV-1 Western Blot Kit			Licensed Kit Total
	Pos	Ind	Neg		Pos	Ind	Neg	
Pos	204	0	0	204	15	0	0	15
Ind	40	8	2	50	1	105	37	143
Neg	0	1	1	2	0	14	38	52
<b>Totals</b>	<b>244</b>	<b>9</b>	<b>3</b>	<b>256</b>	<b>16</b>	<b>119</b>	<b>75</b>	<b>210</b>

In the comparative study on high risk samples, the overall concordance rate was 83% for positive, indeterminate and negative interpretations. The rate of indeterminate test results was 3.5% for this test and 19% for the previously licensed test.

In the comparative study on low risk samples, the overall concordance rate was 75% for positive, indeterminate and negative interpretations. The rate of indeterminate test results was comparable for this test (57%) and the previously licensed test (68%).

Neither test was positive for a sample which was identified as negative by the other test.

To eliminate any bias due to the different interpretive criteria used in the two kits, the comparative data in Table 3 is presented in Table 4 using the same criteria to interpret both tests.

**Table 4: HIV-1 Western Blot Kit and the Previously Licensed Kit Results Interpreted by HIV-1 Western Blot Criteria**

HIV-1 WB Kit Criteria: 2 of 3: gp160/gp120, p24, gp41	WB Result	High Risk EIA RR				Low Risk EIA RR			
		HIV-1 Western Blot Kit			Licensed Kit Total	HIV-1 Western Blot Kit			Licensed Kit Total
		Pos	Ind	Neg		Pos	Ind	Neg	
Previously Licensed kit	Pos	231	0	0	231	16	3	3	22
	Ind	13	8	2	23	0	102	34	136
	Neg	0	1	1	2	0	14	38	52
	<b>Totals</b>	<b>244</b>	<b>9</b>	<b>3</b>	<b>256</b>	<b>16</b>	<b>119</b>	<b>75</b>	<b>210</b>

**Precision**

Reproducibility was evaluated in an in-house study. A panel of eight independent specimens was tested on each of seven separate kit lots. Seven different technicians evaluated bands and interpreted results. The percent of times each band was scored reactive is presented in Table 5 below.

These results demonstrate that for strongly reactive specimens and negative specimens, reproducibility is high. The weakly reactive specimens (ID #s 4 and 5) were less than 100% reproducible for most bands. In these specimens, final result interpretation was not affected.

**Table 5: Reproducibility of the HIV-1 WB Kit**

Specimen	EIA OD	Cutoff	Reactivity	Percent Frequency of Bands								
				gp160	gp120	p65	p55	p51	gp41	p31	p24	p18
1	>2.0	0.232	RR	100	100	100	80	100	100	100	100	100
2	>2.0	0.232	RR	100	100	100	90	100	100	100	100	100
3	>2.0	0.232	RR	100	100	100	100	100	100	100	100	100
4	1.384	0.232	RR	100	90	10	100	10	60	10	100	0
5	0.431	0.232	RR	100	70	100	60	100	100	100	90	0
6	0.026	0.232	Neg	0	0	0	0	0	0	0	0	0
7	0.024	0.232	Neg	0	0	0	0	0	0	0	0	0
8	>2.0	0.232	RR	100	100	100	100	100	100	100	100	100

**INTERFERING FACTORS AND SUBSTANCES**

Testing was performed on specimens from individuals with clinical conditions unrelated to HIV-1 which might result in a reactivity with proteins present. Samples studied included 25 from persons with autoimmune diseases, 12 with elevated gammaglobulins, 110 with viral infections unrelated to HIV-1 and 38 other conditions. The viral infections included samples positive in clinical tests for Cytomegalovirus (12), Infectious Mononucleosis (10), Epstein-Barr virus (3), Rubella (12), Varicella-Zoster (3), Herpes Simplex (12), HBsAg (7), and HTLV-I (39). Although bands were occasionally present at viral locations, none of the strips could be interpreted as positive.

Studies were also performed on 34 samples possessing reactivity to 19 different HLA specificities. Bands were present on nine samples, but only at non-viral locations. Results are presented in Table 6 below.

Further studies were performed on serum and plasma collected in a variety of anticoagulants. Data indicate no interference in interpretation from specimen exposure to anticoagulants. All specimens known to be HIV-1-Positive were interpreted as WB-Positive; all specimens known to be HIV-1-Negative were interpreted as WB-Negative. **No non-viral** bands were observed.

**Table 6: Results of Samples Representing Disease Processes Other than HIV-1 Infection and Other Interfering Factors**

Result	n=	Band Distribution (Number and %)									
		gp160	gp120	p65	p55	p51	gp41	p31	p24	p18	Non-viral
Neg	127 61.4%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
Ind	80 38.6%	0 0.0%	0 0.0%	2 2.5%	6 7.5%	3 3.8%	2 2.5%	0 0.0%	2 2.5%	4 5.0%	73 91.2%
TOTAL	207 100%	0 0.0%	0 0.0%	2 1.0%	6 2.9%	3 1.4%	2 1.0%	0 0.0%	2 1.0%	4 2.0%	73 35.3%

## REFERENCES

1. Gallo RC, *et al.*: Frequent Detection and isolation of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and at Risk for AIDS. *Science* 1984; 224: 500.
2. Montagnier L, *et al.*: A new type of Retrovirus isolated from patients presenting with Lymphadenopathy and Acquired Immune Deficiency Syndrome: Structural and Antigenic Relatedness with Equine Infectious Anemia Virus. *ANN Virol (Inst. Pasteur)* 1984; 135E: 119.
3. Curran JW, Morgan WM, Hardy AM, *et al.*: The epidemiology of AIDS: Current status and future prospects. *Science* 1985; 229:1352-1357.
4. Centers for Disease Control: Antibodies to a retrovirus associated with Acquired Immunodeficiency Syndrome (AIDS) in populations with increased incidences of the syndrome. *MMWR* 1984; 33(27):377-399.
5. Gallo D, Diggs J, Shell G, *et al.*: Comparison of detection of antibody to the acquired immune deficiency syndrome virus by enzyme immunoassay, immunofluorescence and western blot methods. *J Clin Micro* 1986; 23: 1049-1051.
6. Blattner WA, Biggar R, Weiss S, *et al.*: Epidemiology of human T-lymphotrophic virus type III and the risk of the acquired immunodeficiency syndrome. *Ann Internal Med* 1985; 103: 665-70.
7. Watson-Martin P, Burger D, Caouette S, *et al.*: Importance of confirmatory tests after strongly positive HTLV-III screening tests. *N Engl J Med* 1986; 314: 1577.
8. Tsang VCW, Hancock K, Wilson M, *et al.*: Enzyme-linked immunoelectrotransfer blot technique (EITB) (Western blot) for HTLV-III/LAV antibodies. *Developmental Centers for Disease Control, Atlanta*; 1985.
9. Marlink RG, *et al.*: Clinical Hematologic and Immunologic Cross-sectional Evaluation of Individuals Exposed to Human Immunodeficiency Virus Type-2 (HIV-2). *AIDS Res. Human Retroviruses* 1988; 4: 137-148.
10. Update on acquired deficiency syndrome (AIDS). *MMWR* 1982; 31: 507-508.
11. Acquired Immunodeficiency Syndrome (AIDS): Precautions for Clinical and Laboratory Staffs. *MMWR* 1982; 3: 577.
12. Acquired Immunodeficiency Syndrome (AIDS): Precautions for Health-Care Workers and Allied Professionals. *MMWR* 1983; 32: 450.
13. Decontamination of Laboratory Sink Drains to Remove Azide Salts. Centers for Disease Control: Safety Management No. CDC-22, Atlanta; 1976.
14. Interpretation and Use of the Western Blot Assay for Serodiagnosis of Human Immunodeficiency Virus Type 1 Infections. *MMWR* 1989; 38: No. S-7.