

HUMAN IMMUNODEFICIENCY VIRUS TYPE I

GS HIV-1 Western Blot

A Western Blot Kit for the Detection of Antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) in Human Serum, Plasma or Dried Blood Spots.








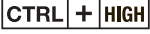





IVD

REF32508 •



40

FOR REFERENCE USE ONLY

LEXICON	
	For In Vitro Diagnostic Use
	Consult Instructions for Use
	Catalog Number
	Lot Number
	Temperature Limit
	Use by (YYYY-MM-DD)
	Low Positive Control
	High Positive Control
	Negative Control
	Number of Tests
	WARNING
	Biohazard
	Manufactured by

NAME AND INTENDED USE

The GS HIV-1 Western Blot Kit is an in vitro qualitative assay for the detection and identification of antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) in human serum, plasma, or dried blood spots. It is intended for use with persons of unknown risk as an additional, more specific test on human serum, plasma, or dried blood spot specimens found to be repeatedly reactive using a screening procedure, such as Enzyme-Linked Immunosorbent Assay (ELISA), and as an additional, more specific test for use with serum, plasma, or dried blood spot specimens obtained from subjects found to be reactive using rapid HIV-1 tests.

SUMMARY AND EXPLANATION OF THE TEST

The major etiologic agent of Acquired Immunodeficiency Syndrome (AIDS) is a retrovirus called Human Immunodeficiency Virus Type 1 (HIV-1).¹⁻³ Enzyme immunoassays (EIA) to detect the presence of viral-specific antibodies to HIV-1 have been described by several investigators. Patients with AIDS and AIDS-related conditions exhibit a high prevalence of antibodies to HIV-1, and antibodies to HIV-1 have also been reported in virus positive, asymptomatic individuals.^{4,5}

The original purpose of HIV-1 screening assays was to detect potentially infectious units of blood and to prevent these units from being used in transfusion or in the manufacture of blood products for transfusion. However, these highly sensitive tests have a relatively low positive predictive value for populations with a low prevalence of HIV-1 infection.⁶ Some specimens may contain antibodies to HLA Class II histocompatibility antigens found on certain cell lines used to produce the virus for commercial applications, or specimens may react with bacterial contaminants associated with production of recombinant proteins.^{7,8} Other individuals who have had no known exposure to HIV-1 react with HIV-1 core proteins in the EIA for unknown reasons.⁹ Since the psychosocial and medical implications of a positive antibody test may be devastating, it is prudent to perform additional testing on such samples to further demonstrate the presence of antibodies specific to HIV-1.

The Western blot as described by Tsang, et. al.,¹⁰ is useful for elucidating the specificity of the antibody response to HIV-1.¹¹⁻¹³ In the Western blot assay, 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 transblotted to a nitrocellulose sheet. The nitrocellulose sheet is then cut into strips, which are reacted with serum, plasma, or dried blood spot specimens.

If virus-specific antibodies are present, they bind to their corresponding viral protein bands. The bands are visualized by using a phosphatase-labeled goat anti-human immunoglobulin conjugate, followed by a substrate for the enzyme. The presence of specific HIV-1 immunoglobulins in serum specimens is indicated by labeling of HIV-1-specific proteins on the strip. Recognized HIV-1 viral antigens produce bands at gp160, gp120, p65, p55, p51, gp41, p40, p31, p24, and p18.

A sample that is repeatedly reactive in the EIA and POSITIVE on the Western blot is presumed to be positive for antibodies to HIV-1.¹⁴ 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.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

Purified, inactivated HIV-1 strain LAV grown in the CEM cell line is disrupted and electrophoretically resolved into bands. The proteins are transblotted onto nitrocellulose sheets, which are cut into strips. Samples are diluted in Specimen Diluent/Wash and applied to the nitrocellulose strip. If specific HIV-1 antibody is present, it binds to proteins resolved on the strip. Unbound sample is removed by washing. The phosphatase-labeled conjugate is then added to the strip and allowed to incubate. The conjugate attaches to antibody already bound to viral proteins on the strip. Excess conjugate is removed by washing. Color Development Reagent is then added to the strip. Reaction sites, where enzyme-labeled antibody is bound, are identified by purple bands. The position and intensity of the bands are compared to reference strips developed using Positive Control sera.

REAGENTS

GS HIV-1 Western Blot Product Description Product No: 32508 (40 Tests)

Component	Contents	Preparation
R1 • HIV-1 Western Blot Strips (40) 2 packages of 20 strips	<ul style="list-style-type: none"> Package contains 20 strips, sufficient for 20 tests; Nitrocellulose strips preblotted with resolved HIV-1 viral proteins Blotting paper buffer contains 0.1% sodium azide and 0.1% ProClin 300 	Ready to use as supplied.
C0 • Western Blot Negative Control 1 vial (0.2ml)	<ul style="list-style-type: none"> Normal human serum or plasma Non-reactive for HBsAg and antibodies to HIV, HCV and HTLV-I/II 0.1% sodium azide 0.5% ProClin 300 	Dilute in Working Specimen Diluent/Wash as described.
C1 • HIV-1 Western Blot Low Positive Control 1 vial (0.2ml)	<ul style="list-style-type: none"> Heat inactivated human serum or plasma containing antibodies reactive to HIV-1 Non-reactive for HBsAg Non-reactive for antibody to HCV and HTLV-I/II 0.1% sodium azide 0.5% ProClin 300 	Dilute in Working Specimen Diluent/Wash as described.
C2 • HIV-1 Western Blot High Positive Control 1 vial (0.2ml)	<ul style="list-style-type: none"> Heat inactivated human serum or plasma containing antibodies reactive to HIV-1 Non-reactive for HBsAg Non-reactive for antibody to HCV and HTLV-I/II 0.1% sodium azide 0.5% ProClin 300 	Dilute in Working Specimen Diluent/Wash as described.
R2 • Western Blot Specimen Diluent/Wash (5X) 2 bottles (100ml)	<ul style="list-style-type: none"> TRIS buffered saline Milk Proteins 0.5% ProClin 300 	Dilute in deionized water as described
R3 • HIV-1 Western Blot Conjugate 1 bottle (80ml)	<ul style="list-style-type: none"> Anti-human IgG, IgA and IgM (Goat) alkaline phosphatase conjugated solution 0.1% sodium azide 0.5% ProClin 300 	Ready to use as supplied.
R4 • Western Blot Color Development Reagent 1 bottle (100ml)	<ul style="list-style-type: none"> 5-bromo-4-chloro-3-indolyl phosphate (BCIP) Nitro blue tetrazolium (NBT) Organic base/TRIS Buffer 	Ready to use as supplied.
Disposable Reaction Trays 8 trays	<ul style="list-style-type: none"> Disposable, slotted reaction trays Lids 	Ready to use as supplied.


Store the kit at 2-8°C upon arrival. All components should be stored at 2-8°C and returned to 2-8°C after use. Bring 5X Specimen Diluent/Wash to room temperature (15-30°C) before use. Return unused strips to package and reseal.

WARNINGS FOR USERS

For *in vitro* Diagnostic Use

WARNING: FDA has licensed this test for use with serum, plasma, and dried blood spot 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. This test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with their potential hazards. Handle appropriately with the requisite Good Laboratory Practices. Wear appropriate protective clothing, including lab coat, eye/face protection and disposable gloves (synthetic, non-latex gloves are recommended) while handling kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
2. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
3. Do not pipette by mouth.
4. GS HIV-1 Western Blot contains human blood components. No known test method can offer complete assurance that infectious agents are absent. Therefore, all human blood derivatives, reagents and human specimens should be handled as if capable of transmitting infectious disease, following recommended Standard and Universal Precautions for bloodborne pathogens as defined by OSHA¹⁵, Biosafety Level 2 guidelines from the current CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories*¹⁶, *WHO Laboratory Biosafety Manual*¹⁷, and/or local, regional, and national regulations.¹⁸ The following human blood derivatives are found in this kit:

- a. The Nitrocellulose strips are preblotted with resolved HIV-1 viral proteins.
- b.  The Normal Human source material (serum or plasma) used in the preparation of the Negative Control (C0) has been tested and found nonreactive for hepatitis B surface antigen (HBsAg) and antibodies to hepatitis C virus (HCV) and human immunodeficiency viruses (HIV-1 and HIV-2).
- c. Human source material containing antibodies reactive to HIV-1, used in the preparation of the Positive Controls (C1 and C2) has been heat-treated. It has been tested and found nonreactive for hepatitis B surface antigen (HBsAg) and antibody to hepatitis C virus (HCV).

Biological spills: Human source material spills should be treated as potentially infectious and immediately decontaminated, including the spill area, materials and any contaminated surfaces or equipment, with an appropriate chemical disinfectant that is effective for the potential biohazards relative to the samples involved (commonly a 1:10 dilution of household bleach, 70-80% Ethanol or Isopropanol, an iodophor [such as 0.5% Wescodyne Plus, EPA Registration # 4959-16-52], or a phenolic, etc.), and wiped dry.¹⁹⁻²¹

NOTE: DO NOT PLACE SOLUTIONS CONTAINING BLEACH INTO THE AUTOCLAVE.

5. The following is a list of potential chemical hazards contained in some kit components (see Section 4 - REAGENTS):

- a. **WARNING:** R1, R2, R3, C0, C1, C2 contain $\leq 0.5\%$ **ProClin 300**.



H317: May cause allergic skin reaction.

P280: Wear protective gloves/protective clothing/eye protection/face protection.

P302 + P 352: IF ON SKIN: Wash with plenty of soap and water.

P333 + P313: If skin irritation or rash occurs: Get medical advice/attention.

P501: Dispose of contents and container in accordance with local, regional, national, and international regulations.

ProClin 300 ($\leq 0.5\%$) is a biocidal preservative that is irritating to eyes and skin, may be detrimental if enough is ingested, and may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.

- b. **WARNING:** R1, R3, C0, C1, C2 contain 0.1% **Sodium azide** (NaN_3).

H303: May be harmful if swallowed.

H313: May be harmful in contact with skin.

P312: Call a POISON CENTER or doctor/physician if you feel unwell.

Sodium azide is a toxic biocidal preservative, which may be harmful in contact with skin or if swallowed; it has been evident to kill at low concentrations, if enough is ingested. Sodium azide may react with certain metals, including lead or copper often found in plumbing, to form highly explosive metal azides. If solutions containing dilute azide are disposed of in the sink after inactivation, flush with copious water to prevent potential explosive build-up. Dispose of product and packaging waste in accordance with all applicable local, regional, national, and international regulations.

The Safety Data Sheets are available at bio-rad.com or upon request.

6. Dispose of all specimens and material used to perform the test as though they contain an infectious agent. Laboratory, chemical or bio-hazardous wastes must be handled and discarded in accordance with all local, regional and national regulations.

PRECAUTIONS FOR USERS

1. Do not use the kit beyond the stated expiration date.
2. Bring Specimen Diluent/Wash to room temperature before use.
3. The only reagents that may be used with different lots of the GS HIV-1 Western Blot are the Specimen Diluent/Wash and Color Development Reagent. Do not mix any other reagents from different lots.
4. Exercise care in opening and removing aliquots from vials to avoid microbial contamination.
5. Prior to removing a strip from the pouch, clean the work surface and forceps with 70% ethanol or isopropyl alcohol.
6. Using forceps, remove each strip from the left side of the series in the package first, and maintain the same order in the reaction trays.
7. For the pipetting of controls and specimens, use individual pipette tips to eliminate carryover of samples.
8. Before measuring samples or reagent, pre-wet the pipette tips with the sample or reagent.
9. Avoid cross-contamination during sample incubation and aspiration:
 - Be particularly careful when transferring reaction trays to the rotator (during sample incubation) to avoid spilling fluid from the troughs.
 - Liquid in the troughs should not contact the tray lid during rotation.

- A strip may be placed in every second trough to further reduce the risk of cross-contamination.
 - Use clean tips to aspirate samples to avoid cross-contamination.
10. Do not re-use trays or lids.
 11. It is important that the pipette used to dispense Working Specimen Diluent/Wash be scrupulously clean. A disposable pipette is preferable.
 12. Use a clean, disposable container (e.g. reagent reservoir) and pipette for dispensing Conjugate. Exposure of Conjugate to serum will inactivate Conjugate.
 13. It is imperative that the final wash, prior to the addition of Color Development Reagent, be performed with deionized or distilled water. Residual Specimen Diluent/Wash in the troughs may decrease band intensity by inhibiting the substrate reaction.
 14. Handle negative and positive controls in the same manner as patient specimens.
 15. If a specimen is inadvertently not added to a strip, the assay result will read negative.
 16. Inadequate adherence to package insert instructions may result in erroneous results.

REAGENT PREPARATION AND STORAGE

A. HIV-1 Western Blot Strips

HIV-1 Western Blot Strips are packed in a resealable plastic pouch between buffer-soaked blotting paper. When ready to use, cut the bag *below* the seal line and retain the upper portion of the bag. Using clean forceps, remove the strips from the bottom portion of the bag. Do not touch the filter paper with gloved hands. Separate the strips to be assayed and place them into troughs of a reaction tray. Place any remaining strips (still inside blotting paper) in the upper portion of the

bag. Seal the bag using the zip closure. Keep tightly sealed. DO NOT LET STRIPS DRY OUT. Store at 2-8°C.

Note: Avoid contaminating the strips or moist blotting paper during handling. This may cause false reactivity.

B. Working Specimen Diluent/Wash

Use Working Specimen Diluent/Wash for wash solution and dilution of serum and plasma samples or elution of dried blood spots. Prepare Working Specimen Diluent/Wash as follows: bring 5X Specimen Diluent/Wash to room temperature, invert to mix before using, and then dilute 1:5 with deionized or distilled water (i.e. 1 part 5X Specimen Diluent/Wash plus 4 parts deionized or distilled water). Clinical laboratory reagent water Type I or Type II is acceptable. For each strip to be tested, prepare 15 ml (i.e., 3 ml 5X Specimen Diluent/Wash plus 12 ml deionized water). Prepare the volume of diluted reagent that will be adequate for the entire run. Mix working solution when combined and again just prior to use. The Working Specimen Diluent/Wash may be stored at 2-8°C for up to two weeks. Bring to room temperature and mix thoroughly prior to use.

Preparation of Working Specimen Diluent/Wash

Number of Strips to be used	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Amount of 5X Spec. Dil/Wash (ml)	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60
Deionized Water (ml)	48	60	72	84	96	108	120	132	144	156	168	180	192	204	216	228	240
Total Volume (ml)	60	75	90	105	120	135	150	165	180	195	210	225	240	255	270	285	300

SPECIMEN COLLECTION, PREPARATION AND STORAGE

Serum or Plasma

Serum or plasma may be used. The following anticoagulants have all been evaluated and found to be acceptable: EDTA, heparin, sodium citrate, CPDA-1, and ACD. Samples which are collected into anticoagulant tubes should be filled as labeling indicates to avoid improper dilution. Specimens with observable particulate matter should be clarified by centrifugation prior to testing. No clinically significant effect has been detected in assay results with increased levels of protein, lipids, bilirubin, or hemolysis, or after heat inactivation of patient samples.

Serum or plasma may be stored at 2-8°C for up to seven days. Samples should not be used if they have incurred more than 5 freeze/thaw cycles. Mix samples thoroughly after thawing.

If specimens are to be shipped, they should be packed in compliance with Federal Regulations covering the transportation of etiologic agents. Studies have demonstrated that specimens may be shipped refrigerated (2-8°C) or at ambient temperatures ($\leq 37^{\circ}\text{C}$) for up to 7 days. For shipments that are in transit for more than 7 days, specimens should be kept frozen (-20°C or lower). Refrigerate samples at 2-8°C at receipt, or freeze for longer storage.

Collection of Dried Blood Spots

In addition to detecting antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) in human serum or plasma, the GS HIV-1 Western Blot may be used to test whole blood specimens collected onto filter paper and dried. Drops of whole blood should be obtained by using a licensed collection kit or collected according to the National Committee for Clinical Laboratory Standards,¹⁸ by either finger puncture or heel stick.

1. Label a separate piece of filter paper for each specimen with the appropriate specimen identification. Use a ball point pen or other water-indelible marker. Handle the filter paper by the edges; do not touch the areas that will be used to collect specimens.

2. Prepare the area (either finger or heel) for puncture. The puncture must be performed with sufficient force and penetration to sustain a flow of at least several drops of blood. Allow a large drop of free flowing blood to collect at the puncture site. Touch the filter paper to the edge of the drop to collect the drop, and allow another large drop to form at the puncture site. Continue to collect drops in this manner until the wound ceases to bleed or until collection is sufficient.
3. Collect each drop of blood in a separate area of the filter paper (if the paper is marked with several circles, place each drop in a different circle). Do not layer successive drops of blood in the same spot. In addition to the sample that is required to perform the EIA (i.e., one spot of blood $\geq 1/4$ of an inch in diameter), at least one spot of blood that is $\geq 1/4$ of an inch in diameter must be obtained in order to perform the GS HIV-1 Western Blot.
4. If the wound stops flowing before sufficient blood has been obtained, a second puncture should be performed. The wound may be massaged very gently to encourage formation of large blood droplets. Do not squeeze the wound to obtain more blood as this may result in hemolysis of the specimen or a mixture of other body fluids with the specimen.
5. After the blood has been absorbed into the filter paper, it should be dried at room temperature for at least three hours. The filter paper may be allowed to dry at room temperature overnight. When dry, the spots will be a uniform dark brown. No areas of red coloration should be seen; the appearance of the spots should be similar to that of a dried blood stain.
6. When the blood spots are completely dry, a sample may be punched and eluted as described below (See Specimen Handling and Preparation). If dried blood specimens are to be shipped, they should be enclosed and sealed in either a moisture barrier container, such as a heavy duty zip-lock bag with desiccant,²² or a high-quality bond envelope.²³

Storage of Dried Blood Spots

Studies performed indicate that completely dried specimens may be stored frozen (-20°C) or refrigerated (2-8°C) for at least two months under low humidity conditions. Storage under accelerated temperature conditions (room temperature or 37°C) leads to increasing non-specific reactivity over time. Therefore, it is recommended that dried blood spots be stored for no more than one week at room temperature, or up to two months refrigerated or frozen; routine storage at room temperature or higher is not recommended. If specimens are stored at any conditions other than the ones listed above, the user must validate the stability of the specimens under those storage conditions. If specimens are to be stored in a humid environment ($\geq 60\%$ relative humidity), the user should include a desiccant.

This kit is not licensed for use with specimens other than serum, plasma, and dried blood spots. This kit is not intended for use on saliva/oral fluids or urine samples.

GS HIV-1 WESTERN BLOT PROCEDURE

Materials Provided

See Reagents Section on Page 3.

Materials Required But Not Provided

1. Disposable Pipettes.
2. Calibrated precision micropipettes to deliver variable volumes from 5 - 1000 μl , (accurate within $\pm 5\%$).
3. Disposable pipette tips.
4. Rotary platform capable of rotating at 50 - 60 rpm.
5. Aspirator.
6. Plastic forceps for strip handling.
7. Glass or plastic container for preparation of Working Specimen Diluent/Wash, 15 - 600 ml.

- Clean, disposable container, such as a reagent reservoir, for dispensing Conjugate.
- Deionized or distilled water. Clinical laboratory reagent water Type I or Type II is acceptable.
- Gloves.
- Laboratory timer.

Preliminary Statements

- The expected run time for this procedure is approximately 3 hours. Each run of this assay must proceed to completion without interruption after it has been started.
- Positive and Negative Controls must be evaluated with each run. Compare intensity of patient samples to controls for each set of strips to determine patient results.
- The minimum controls to be included in each run of this assay are: one HIV-1 Low Positive Control, one HIV-1 High Positive Control, and one Negative Control. If multiple packages of strips are used in one run, controls must be included for each package of strips.**
- Do not splash controls, specimens, or reagents between troughs of the reaction tray.
- Cover reaction trays for each incubation step using lid provided, or equivalent.
- Ensure that each one of the strips is entirely covered with liquid during the incubation steps.
- Adhere to the recommended time constraints for the incubation steps.

SPECIMEN HANDLING AND PREPARATION

Caution: Handle all specimens, controls, and HIV-1 Western Blot Strips as though capable of transmitting infectious disease.

Dried Blood Spot Specimen Preparation

1. Use a 1/4" paper punch to remove a 1/4" disk of each whole blood specimen to be tested. Punch the disk from a uniform area of one of the completely dried spots of blood. Place each 1/4" disk in a separate, clean, empty trough of a reaction tray.
2. Use a precision pipette or repeating dispenser to add 500 μ l of Working Specimen Diluent/Wash to each trough containing a filter paper disk. Mix the specimens well to wet the filter paper thoroughly (for example, use a rotator). Cover the reaction tray with a lid to minimize evaporation. Tilt tray approximately 5/8" (15 mm) to concentrate liquid in area of spot. Elute the specimens 2 hours at room temperature with rotation at 50 - 60 rpm, or overnight (static) at 2-8°C.
3. At the end of the two hour elution or when the refrigerated eluates warm to room temperature, the filter paper disk should be almost white (a faint brown color may remain in the disk). Specimens eluted in this manner are ready to use without further dilution in the GS HIV-1 Western Blot. The filter paper disk may be removed from the trough with clean forceps before starting the assay, or the filter paper disk may be left in the trough and removed just prior to the addition of Conjugate (before step 7 of the Procedure). Clean the forceps with deionized water and wipe dry in between removal of each filter paper disk in order to avoid cross-contamination.

Wash Procedure for Serum, Plasma or Dried Blood Spots

Repeat this procedure four times after the primary (sample) and secondary (conjugate) incubations. Incomplete or ineffective washing will compromise the assay. **DO NOT ALLOW THE STRIPS TO DRY OUT.**

1. **Aspirate the liquid from the troughs.** Wash aspiration manifold (if used) by aspirating water for 3 - 5 seconds between trays of strips. **Immediately fill each trough** with at least 1 ml Working Specimen Diluent/Wash.
2. **Rock the reaction tray(s)** back and forth approximately 8 times. Be certain that each strip is immersed in liquid and is moving freely when rocked.
3. **Aspirate the liquid completely.** Wash aspiration manifold (if used) by aspirating water for 3 - 5 seconds between trays of strips.

ASSAY PROCEDURE

1. Prior to the start of the assay, prepare Working Specimen Diluent/Wash (see page 9, step B). **Allow to come to room temperature before use.**
2. Label the reaction trays appropriately to maintain control and sample identification.
3. **Add diluted specimens to reaction trays.**
 - **Serum and Plasma:** Using a clean, disposable pipette, carefully add 1 ml Working Specimen Diluent/Wash into each trough. Using a clean, disposable, precision micropipette, transfer 10 μ l of each control or specimen to the respective trough containing 1 ml of Working Specimen Diluent/Wash (final dilution 1:101).
 - **Dried Blood Spots:** See Dried Blood Spot Specimen Preparation, page 14. Note: the required volume of dried blood spot eluate is 0.5 ml/strip.
4. **Place one strip, with the *indicator line facing up*, into each trough containing a diluted serum or plasma specimen, dried blood spot eluate, or control.**

Note: To avoid cross-contamination of the strips, the following instructions should be followed.

- a. While wearing a fresh pair of gloves, cut the strip pouch below the heat seal with cleaned scissors, leaving the zipper seal intact.
 - b. Trim the side edges of the strip pouch around the filter paper, leaving the bottom (folded) edge intact.
 - c. Do not touch the filter paper with anything but cleaned forceps. Leave the outer plastic in place to hold the pouch, and use cleaned forceps when manipulating filter paper and strips.
 - d. Gently detach each strip with forceps at the top of the strip (near the number).
 - e. Transfer the strip carefully; avoid contact with contaminated surfaces. Do not touch the forceps to the liquid in the trough.
 - f. Keep unused strips and filter paper in the original plastic folder. Store the plastic folder in the zipper sealed bag.
5. Cover the reaction tray with the lid and **incubate with gentle agitation** on a rotator at 50-60 rpm for 60-65 minutes at room temperature.

Note: Be certain that each strip is immersed in the liquid and is moving freely during rotation. Liquid should not contact the tray lid during rotation.

6. After incubation, aspirate the liquid from each trough. **Wash the strips at least four times** with Working Specimen Diluent/Wash (refer to the Wash Procedure). After the final wash, add 1.0 ml Working Specimen Diluent/Wash to each trough. Place the reaction tray on the rotator for 4-6 minutes.

Note: If filter paper disks have not already been removed, remove them now with forceps. Clean the forceps with deionized water and wipe dry between removal of each filter paper disk in order to avoid cross contamination.

7. Following the soak, aspirate the contents of each trough completely. **Immediately add 1.0 ml Conjugate to each trough.**

- Cover the reaction tray with the lid and **incubate with gentle agitation** on the rotator at 50-60 rpm for 45-50 minutes at room temperature.

Note: Be certain that each strip is immersed in the liquid and is moving freely during rotation. Liquid should not contact the tray lid during rotation.

- After incubation, aspirate the liquid from each trough. **Wash the strips at least four times** with Working Specimen Diluent/Wash (refer to the Wash Procedure). After the final wash, **add 1.0 ml deionized water** to each trough. Place the reaction tray on a rotator for 4-6 minutes.
- Following the soak, aspirate the deionized water from each trough completely. **Immediately add 1.0 ml Color Development Reagent** to each trough. Gently rock the reaction tray back and forth at least twice. Be sure that each strip is immersed in Color Development Reagent. **Allow color to develop for 3-4 minutes. Do not over-incubate Color Development Reagent.**
- Stop the reaction** by aspirating the Color Development Reagent and flooding each trough with a minimum of 1.0 ml deionized water, aspirate, and repeat the addition of 1.0 ml deionized water. Allow to stand for 5-10 minutes. Aspirate the water.
- Allow the strips to **air dry** before reading the bands. Handle the strips carefully. Use forceps to remove the strips from the troughs.
- Store completed strips away from direct light to avoid fading. For permanent storage, it may be desirable to mount the strips on durable paper and cover them with a plastic sheet for protection.

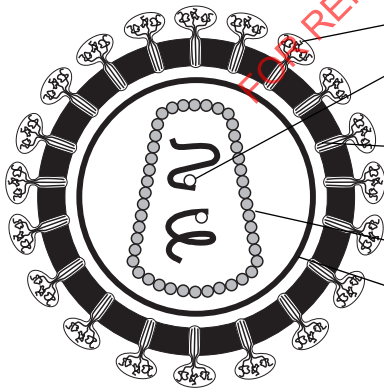
QUALITY CONTROL

For the results of the assay to be considered valid, the following conditions must be met:

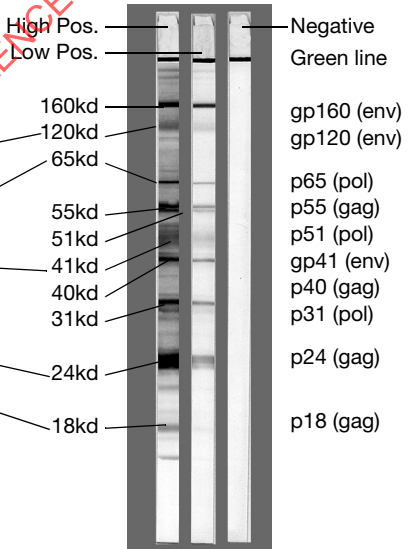
- Negative Control** No bands should be observed.
- HIV-1 Low Positive Control** A band must be present at gp120, and the interpretation of the Low Positive Control must be positive. Other bands may or may not be present.
- HIV-1 High Positive Control** Bands must be present at gp160, gp120, gp41 and p24. Other bands may or may not be present.

The CDC/ASTPHLD criteria are used for result interpretation.²⁴ The major bands of diagnostic significance on a Western blot reactive for HIV-1 are gp160, gp120, gp41, and p24. Other bands may also be present. Note: the p51 and p55 bands may co-migrate and can be interpreted as one p51/p55 entity.

ANATOMY OF HIV-1



HIV-1 ASSOCIATED BANDS



INTERPRETATION OF RESULTS

Evaluate each strip for the presence of bands. A band is defined as a distinct purple line which extends horizontally across the strip where human antibodies have bound to resolved proteins. The lines may be of varying thicknesses. Smudges, spots, and lines which do not extend the width of the strip should not be interpreted as bands. Any lines that are higher than gp160 or lower than p18 on the strip are outside of the reading area of the strip and therefore are not to be interpreted as bands.

Use the HIV-1 High and Low Positive Controls to identify bands which may be present on patient specimen strips. Score relative intensity of bands present on patient specimen strips by comparing to bands on control strips as follows:

Intensity of Band	Score
• Absent	-
• Less than the intensity of gp120 on the HIV-1 Low Positive Control strip.	+/-
• At least as intensely reactive as gp120 on the HIV-1 Low Positive Control strip but less intense than gp120 on the HIV-1 High Positive Control strip.	+
• Greater than or equal to the intensity of gp120 on the High Positive Control strip.	++

Interpret the immunoblot as NEGATIVE, INDETERMINATE, or POSITIVE based on the pattern that is present.

POSITIVE

At least TWO of the major bands: gp160 and/or gp120, gp41, or p24 must be present. (The presence of gp160 and/or gp120 qualifies as one major band.) Bands must be at least as intense as the Low Positive Control gp120 band (a reactivity score of + or greater) to be considered POSITIVE. The band at gp41 must be broad and diffuse.

INDETERMINATE

One or more bands are present but the blot does not meet the criteria for a POSITIVE result as described above.

NEGATIVE

No bands are present.*

*Note: Negative dried blood spot specimens frequently exhibit a weakly reactive (+/-) fine band migrating within the wide gp41 region. This reactivity is clearly distinguishable from gp41, which is a broad, diffuse band. Dried blood spot specimens that are reactive only with this discrete "p42" band or contain only lines or marks that are higher than gp160 or lower than p18 may be interpreted as NEGATIVE.

INDETERMINATE results should not be considered either POSITIVE or NEGATIVE. Additional immunoblot testing and clinical evaluation must be utilized to correctly evaluate an INDETERMINATE result.

LIMITATIONS OF THE PROCEDURE

NOTE: Only a serum or plasma sample should be used to test blood or plasma intended for transfusion or further manufacture.

1. The assay must be performed in strict accordance with these instructions to obtain reproducible results.
2. Although a persistently POSITIVE immunoblot for antibodies to HIV-1 indicates infection with the virus, a diagnosis of Acquired Immunodeficiency Syndrome or AIDS can only be made on clinical grounds if an individual meets the case definition of AIDS established by the Centers for Disease Control.²⁵

3. Individuals with POSITIVE immunoblots for antibodies to HIV-1 should be referred for medical evaluation which may include additional testing. The clinical implications of antibodies to HIV-1 in an asymptomatic individual are not known. However, a large proportion of such individuals have virus detectable in their blood cells and some such individuals will develop immunodeficiency with the passage of time.²⁶
4. It is generally recognized that detection of HIV antibody in infants born to seropositive mothers is not adequate to diagnose HIV infection in the infant, since maternal IgG frequently persists for as long as 18 months after birth. Supplemental assays designed specifically for neonatal specimens may be helpful in resolving such cases.²⁷
5. INDETERMINATE immunoblots should not be used as the sole basis of diagnosis of HIV-1 infection. However, such findings may provide useful information in the context of medical evaluation in which clinical information is available.
6. Persons with an INDETERMINATE immunoblot should be retested using a fresh specimen after six months.
7. A NEGATIVE immunoblot does not exclude the possibility of infection with HIV-1. Antibody testing should not be used in lieu of blood donor exclusion and self-exclusion procedures.
8. Strips which are obscured by the development of dark blotches or marks should not be interpreted.
9. 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.

PERFORMANCE CHARACTERISTICS

The performance of the GS HIV-1 Western Blot was evaluated in clinical studies in low risk, high risk, and AIDS populations. Samples were tested with licensed HIV-1/HIV-2 EIA test kits, FDA licensed HIV-1 Western Blot kits, and with the GS HIV-1 Western Blot. A total of 1430 sera/plasma samples (1115 serum; 315 plasma) and 345 dried blood spots (DBS) were tested by 7 independent clinical sites located in both high and low prevalence areas for HIV-1.

Reproducibility

The reproducibility of the GS HIV-1 Western Blot was evaluated at 7 sites with a panel of 6 specimens tested in duplicate on 5 runs on each of 3 lots. Each band was scored independently by 2 readers at each site. The percent of times each band was scored as present is shown in Table 1.

**Table 1: Precision
GS HIV-1 Western Blot**

Panel ID	% Frequency of Bands Present (number \geq +/-)								
	gp160	gp120	p65	p55/51	gp41	p40	p31	p24	p18
Negative Normal Donor	0%	0%	0.5%	0.5%	0%	0%	0%	0%	0%
Negative Pooled Plasma	0%	0%	1.0%	1.0%	0.5%	0%	1.4%	2.4%	1.0%
HIV-2 Positive Sample	1.0%	0%	95.7%	100%	45.7%	100%	99.0%	100%	4.8%
Weak HIV-1 Positive Sample	96.2%	1.4%	91.9%	70.5%	2.9%	100%	77.6%	100%	2.9%
HIV-1 High Positive Sample	100%	100%	100%	100%	100%	100%	100%	100%	99.5%
HIV-1 Low Positive Sample	100%	100%	100%	100%	100%	100%	100%	100%	93.8%

SENSITIVITY AND SPECIFICITY

Serum/Plasma

A study in low risk populations included 298 sera/plasma collected prospectively from normal blood donors, as well as 274 EIA repeatedly reactive sera/plasma selected retrospectively from blood center repositories (Table 2). This latter group of samples was chosen to include sera/plasma testing positive or indeterminate on a licensed HIV-1 Western Blot, as well as unselected with regard to reactivity on a licensed HIV-1 Western Blot test.

The GS HIV-1 Western Blot was positive for 63/63 (100%) blood center repository samples identified as positive by the licensed HIV-1 Western Blot, demonstrating comparable sensitivity. The GS HIV-1 Western Blot was negative for 68/78 (87.2%) samples found to be negative by the licensed HIV-1 Western Blot, demonstrating comparable specificity. (The GS HIV-1 Western Blot was indeterminate for the remaining 10 samples).

Of the 298 prospective normal donor samples, all were EIA non-reactive. The GS HIV-1 Western Blot was negative for 266 (89.3%) samples, indeterminate for 32 (10.7%) samples, and positive for none (0.0%). Twenty-six (26) of the 32 indeterminate samples were additionally tested with a licensed HIV-1 Western Blot, and found to be either indeterminate or negative (Table 2).

Table 2: Evaluation of the GS HIV-1 Western Blot in Low Risk Populations (n=572)

Clinical Group	GS HIV-1 Western Blot Interpretation	Licensed Western Blot Interpretation (historical data)		
		Positive	Indeterminate	Negative
Donors EIA Repeatedly Reactive (Retrospective Study) (n=274)	Positive (n=63)	63	0	0
	Indeterminate (n=83 ^a)	0	73	10
	Negative (n=128)	0	60 ^b	68
Donors EIA Non-Reactive (Prospective Study) (n=298)	Positive (n=0)	0	0	0
	Indeterminate (n=32 ^c)	0 ^c	12 ^c	14 ^c
	Negative (n=266 ^d)	0 ^d	4 ^d	17 ^d

^a One sample was originally entered into the study as Western blot positive (historical data) and found to be indeterminate on GS HIV-1 Western Blot. It was also indeterminate when retested with the licensed Western blot suggesting that the sample may have deteriorated during storage.

^b These were repository specimens that had been stored frozen for various periods of time. Results of GS HIV-1 Western Blot are compared to historical licensed HIV-1 Western Blot data. No follow-up studies were done to determine if the observed differences were due to sample deterioration during storage.

^c Six (6) of the samples that were indeterminate on the GS HIV-1 Western Blot were not tested on the licensed Western blot.

^d Two hundred forty-five (245) of the 266 samples Negative on the GS HIV-1 Western blot were not tested on the licensed Western blot.

Additional studies in HIV high risk and AIDS populations included 525 samples collected prospectively and retrospectively (Table 3). Prospectively collected samples included 152 sera from AIDS patients; 20 sera from ARC patients; 26 sera from HIV-1 asymptomatic patients; and 199 repository sera from high risk subjects. These included samples collected in a sequential manner from STD clinic patients (n = 49); homosexual males (n = 50); and hospital emergency room patients (n = 100) in a high prevalence area. Retrospective samples included 128 repository specimens testing EIA repeatedly reactive and positive on a licensed HIV-1 Western Blot. These samples were from various geographic loca-

tions including France (n = 12), Ghana (n = 5), Nairobi (n = 7), Nigeria (n = 12), Australia (n = 12), Thailand (n = 12), Zimbabwe (n = 12), Sierra Leone (n = 12), Mozambique (n = 2), Central African Republic (n = 14) and the USA (n = 28).

These studies demonstrated 172/172 (100%) positive results in AIDS and ARC populations and 176/176 (100%) positive results for high risk samples confirmed positive on a licensed HIV-1 Western Blot. Overall, there was 100% concordance of positive results between the GS HIV-1 Western Blot and the licensed Western blot. Of the 176 EIA non-reactive specimens from high risk populations, 8 were indeterminate and 137 were negative on both tests, demonstrating 82.4% concordance. There were 31 discordant results, all of which were indeterminate or negative on the two Western blot kits, as shown in Table 3.

Table 3: Comparison of the GS HIV-1 Western Blot with a Licensed HIV-1 Western Blot in AIDS, ARC, and High Risk Populations (n=525)

Clinical Group	GS HIV-1 Western Blot Interpretation	Licensed Western Blot Interpretation		
		Positive	Indeterminate	Negative
AIDS/ARC ^a EIA Repeatedly Reactive (n=172)	Positive (n=172)	172	0	0
	Indeterminate (n=0)	0	0	0
	Negative (n=0)	0	0	0
High Risk ^b EIA Repeatedly Reactive (n=177)	Positive (n=176)	176	0	0
	Indeterminate (n=1)	0	1	0
	Negative (n=0)	0	0	0
High Risk ^c EIA Non-Reactive (n=176)	Positive (n=0)	0	0	0
	Indeterminate (n=28)	0	8	20
	Negative (n=148)	0	11	137

^a AIDS (n =152); ARC (n = 20)

^b HIV-1 Asymptomatic (n = 26); Prospective high risk (n = 23); HIV-1 confirmed positives from different geographic locations (n = 128)

^c Prospective high risk (n = 176)

Additional testing of GS HIV-1 Western Blot was performed on 333 specimens from persons with clinical conditions unrelated to HIV-1 that might result in a reactivity with proteins present. Table 4 summarizes these results. Although weak bands were occasionally present for viral proteins, none of the samples would meet the package insert requirements for an interpretation of POSITIVE.

Table 4: GS HIV-1 Western Blot Results for Specimens from Patients with Conditions Unrelated to HIV-1 (n = 333)

Clinical Condition	% Frequency of Bands Present (number \geq + by one or both readers)										
	Number Studied	Number with Bands	gp160	gp120	p65	p55/51	gp41	p40	p31	p24	p18
Toxoplasmosis	20	2	0	0	0	0	0	0	0	1	1
Rheumatoid factor	18	3	1	0	0	0	0	0	0	0	2
ANA	9	0	0	0	0	0	0	0	0	0	0
SLE	8	0	0	0	0	0	0	0	0	0	0
Elevated IgG	20	6	1*	0	0	1	1*	0	2	3*	1
Elevated IgM	20	5	0	0	0	0	0	0	1	4	0
Anti-HBV +	20	2	0	0	0	0	0	1	0	2	0
Anti-HTLV-I +	10	0	0	0	0	0	0	0	0	0	0
Anti-HTLV-I/II +	4	1	0	0	0	0	0	1	0	1	0
Anti-HTLV-II +	6	0	0	0	0	0	0	0	0	0	0
Anti-CMV +	19	2	0	0	1	0	0	0	0	0	1
Anti-EBV +	20	3	0	0	0	3	0	0	0	0	0
Anti-HAV IgM +	10	0	0	0	0	0	0	0	0	0	0
Anti-HAV Total +	10	1	0	0	0	0	0	0	0	1	0
Anti-HCV +	20	1	0	0	0	0	0	0	1	0	0
Anti-HSV +	20	2	0	0	0	0	0	0	0	2	0
Anti-Rubella +	19	0	0	0	0	0	0	0	0	0	0
Cancer	20	2	0	0	0	0	0	1	0	2	0
Multi-Transfusion	20	2	0	0	0	0	0	0	1	0	1
Multiparous women	20	0	0	0	0	0	0	0	0	0	0
Cirrhosis (type unknown)	20	1	0	0	0	0	0	0	0	1	0

*None were interpreted as Western blot positive.

Dried Blood Spots

In a comparative study to demonstrate equivalent performance of dried blood spots (DBS) compared to serum or plasma on the GS HIV-1 Western Blot, a total of 345 DBS paired to sera/plasma from low risk, high risk, and AIDS populations were tested at 5 clinical sites. The dried blood spots were tested with the licensed Genetic Systems™ HIV-1/HIV-2 EIA (DBS Procedure) and with the GS HIV-1 Western Blot. Results were compared to the testing results of the paired sera/plasma on the GS HIV-1 Western Blot.

AIDS and high risk samples (Table 5) included a total of 248 paired sera and dried blood spot samples: 152 paired samples from AIDS patients; 20 paired samples from ARC patients; 26 paired samples from HIV-1 asymptomatic patients; and 50 simulated dried blood spots prepared with 50 prospective high risk sera. [Note: The simulated DBS were prepared by mixing equal volumes of serum and washed packed red blood cells (Type O, certified to be non-reactive for HIV and HBsAg)].

Paired dried blood spots demonstrated 172/172 (100%) positive results in AIDS and ARC populations and 26/26 (100%) positive results for HIV-1 asymptomatic patients. Of the 50 prospective high risk samples, 20 DBS and paired sera were EIA repeatedly reactive and 30 DBS and paired sera were EIA non-reactive. Twenty-seven (27) dried blood spots were negative, 3 were indeterminate and 20 were positive when tested with the GS HIV-1 Western Blot. By comparison, the 27 paired sera were negative, the 3 were indeterminate and the 20 were positive when tested with the GS HIV-1 Western Blot, demonstrating equivalence in sensitivity and specificity (concordance = 100%) for the testing of serum and dried blood spots.

Table 5: Comparison of Results for Paired Sera and Dried Blood Spots with GS HIV-1 Western Blot in AIDS, ARC, and High Risk Populations (n=248)

Clinical Group	Licensed HIV-1/HIV-2 EIA	GS HIV-1 Western Blot Interpretation		
		Positive	Indeterminate	Negative
AIDS, ARC, High Risk Serum	EIA Repeatedly Reactive (n=218)	218	0	0
	EIA Non-Reactive (n=30)	0	3	27
AIDS, ARC, High Risk Dried Blood Spots	EIA Repeatedly Reactive (n=218)	218	0	0
	EIA Non-Reactive (n=30)	0	3	27

Low risk samples included 97 DBS paired to 97 sera/plasma from normal blood donors (Table 6). Of these, 96 DBS were EIA non-reactive and 1 was EIA repeatedly reactive. By comparison, none of the sera/plasma were EIA reactive. Sixty-three (63) DBS were negative, 34 were indeterminate and none were positive on the GS HIV-1 Western Blot. In testing the paired sera/plasma from these same normal donors, 78 were negative, 19 were indeterminate, and none were positive on the GS HIV-1 Western Blot [concordance = $(15 + 59)/97 = 76.3\%$] demonstrating comparable specificity for the testing of serum/plasma and dried blood spots.

Table 6: Comparison of Results for Paired Sera/Plasma and Dried Blood Spots with GS HIV-1 Western Blot for Normal Blood Donors (n=97)

GS HIV-1 Western Blot Interpretation Serum or plasma	GS HIV-1 Western Blot Interpretation DBS		
	Positive (n=0)	Indeterminate (n=34)	Negative (n=63)
Positive (n=0)	0	0	0
Indeterminate (n=19)	0	15	4
Negative (n=78)	0	19	59

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