

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

 **OraSure**<sup>®</sup>

**HIV-1 Western Blot Kit**

An Enzyme Immunoassay for the Detection of Antibodies  
to Human Immunodeficiency Virus Type 1 (HIV-1)  
in Human Oral Fluid Specimens Obtained with  
OraSure<sup>®</sup> HIV-1 Oral Specimen Collection Devices

20 Tests

Store at 2-8°C

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## NAME AND INTENDED USE

The OraSure® 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 oral fluid specimens obtained with the OraSure HIV-1 Oral Specimen Collection Device. For convenience, the collection device will hereafter be referred to as “OraSure”. The OraSure HIV-1 Western Blot Kit is intended for use as an additional, more specific test for HIV-1 antibodies in OraSure specimens collected from individuals of unknown risk for HIV-1, which are found to be repeatedly reactive by the Oral Fluid Vironostika® HIV-1 Microelisa System. The OraSure HIV-1 Western Blot Kit is intended for professional use only.

The OraSure HIV-1 Western Blot Kit is not intended for use with blood, serum/plasma or urine specimens or for screening potential blood donors.

## 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> Published data have established that patients with AIDS and individuals infected with HIV-1 produce antibodies against HIV-1 proteins. Studies have shown that HIV-1 antibodies have been detected in saliva samples of most HIV-infected patients.<sup>5-7</sup>

A number of EIA kits are currently available for the screening of serum specimens for HIV-1 antibodies. The Oral Fluid Vironostika HIV-1 Microelisa System manufactured by bioMérieux, Inc. is available specifically for screening OraSure oral fluid specimens. These specimens are obtained using the OraSure HIV-1 Oral Specimen Collection Device which is designed to collect oral HIV-1 antibodies while minimizing problems inherent in saliva samples (namely in high viscosity and instability). Samples found to be repeatedly reactive for HIV-1 antibodies are tested using additional, more specific tests, such as Western blot or immunofluorescence assays. 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 (a summary of the principles of the assay is presented on page 2).

Clinical serum samples that are reactive in the screening assays but do not contain HIV-1 antibodies have also been described.<sup>9</sup> Some of these samples possess antibodies to certain Class II HLA histocompatibility antigens that are found in some cell lines used to produce the virus. Other individuals, who have had no known exposure to HIV-1, produce reactive results in the screening test for unknown reasons. Such nonspecific results are found commonly when screening tests are used in low risk populations. Since the psychosocial and medical implications of a positive antibody test may be significant, it is recommended that additional testing be performed on such samples to validate the presence of antibodies specific to HIV-1.<sup>10</sup>

The OraSure HIV-1 Western Blot Kit was developed in order to provide an additional, more specific assay for HIV-1 antibody detection in OraSure specimens found to be repeatedly reactive in the screening EIA. The OraSure HIV-1 Western

Blot Kit, when used as directed in this insert, will detect antibodies to HIV-1 when present in human oral fluid samples obtained with the OraSure HIV-1 Oral Specimen Collection Device. The position of bands on the preblotted nitrocellulose strips allows the antibody reactivity to be associated with specific viral antigens. An OraSure sample that is reactive in both EIA screening test and Western blot assay is presumed to be positive for antibodies 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>11</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). Absence of antibodies to HIV cannot be taken as absolute proof that an individual is free of HIV-1 or incapable of transmitting the virus. Individuals with positive tests should be referred for medical evaluation.

### **CHEMICAL AND BIOLOGICAL PRINCIPLES OF THE PROCEDURE**

The whole cell viral lysate used in the manufacture of the OraSure HIV-1 Western Blot Kit is manufactured by bioMérieux, Inc. (U.S. license number 956). It is HIV-1 propagated in an H-9/HTLV-III<sub>B</sub>, T-lymphocyte cell line. It is purified by ultracentrifugation 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.

OraSure 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, alkaline phosphatase-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 a substrate specific to the alkaline phosphatase. 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, purple 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)

**OraSure® HIV-1 Western Blot Strips** **20 strips**  
Prenumbered nitrocellulose strips, preblotted with resolved HIV-1 proteins; packed in a resealable plastic pouch between buffer-soaked absorbent paper; buffer contains 0.1% sodium azide as a preservative.

**Substrate** **1 bottle; 22 mL**  
BCIP/NBT single reagent substrate in an organic base/TRIS buffer.

**Powdered Milk** **1 bottle; 30 g**  
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; 0.25 mL**  
Goat anti-human IgG (heavy and light chains) F(ab')<sub>2</sub> fragment, labeled with alkaline phosphatase; contains 0.1% sodium azide as a preservative.

**OraSure® HIV-1 WB Negative Control** **1 vial; 0.65 mL**  
Human serum or plasma, nonreactive for antibodies to HIV-1, in OraSure Control Matrix; tested negative for HBsAg and antibodies to HCV; contains a proprietary preservative.

**OraSure® HIV-1 WB Low Positive Control** **1 vial; 0.65 mL**  
Human serum or plasma, reactive for antibodies to HIV-1, in OraSure Control Matrix; tested negative for HBsAg and antibodies to HCV; heat-inactivated to render material noninfectious for HIV-1; contains a proprietary preservative.

**OraSure® HIV-1 WB High Positive Control** **1 vial; 0.65 mL**  
Human serum or plasma, reactive for antibodies to HIV-1, in OraSure Control Matrix; tested negative for HBsAg and antibodies to HCV; heat-inactivated to render material noninfectious for HIV-1; contains a proprietary preservative.

**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>12</sup>

## EQUIPMENT REQUIRED BUT NOT SUPPLIED

1. Centrifuge tube for each sample to be processed
2. Centrifuge
3. Refrigerator (2-8°C)
4. 37°C water bath
5. Graduated cylinders
6. Beaker or appropriate mixing vessel
7. Balance
8. Laboratory timer
9. Magnetic stir plate and stir bar
10. Test Tubes: glass, polypropylene, or polystyrene (12x75 mm) (optional)
11. Precision micropipets to deliver variable volumes from 5 to 1000 µL
12. Disposable pipet tips
13. Graduated pipets to deliver volumes to 25 mL
14. Scissors
15. Forceps for strip handling (plastic or Teflon-coated)
16. Transfer pipets
17. Rotary platform, capable of rotating at 50-60 rpm
18. Aspiration system
19. Repeating pipet to deliver 1-2 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  
Store at room temperature while performing assay. 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 five minute wash and applied immediately after the wash is complete and the wash solution is aspirated. Excess Conjugate dilution must be discarded.
  - d. All other kit components are supplied ready to use.
4. Keep strip pouch tightly sealed. *Do not let strips dry out.*

## CHEMICAL OR PHYSICAL INDICATIONS OF INSTABILITY

Alterations in 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 8, step 1.a).

Substrate is pale yellow in color. A fine black precipitate may be observed but its presence does not affect product performance.

## SPECIMEN COLLECTION, STORAGE AND PREPARATION

**Note:** This test kit may not be used to assay blood specimens. This test kit may only be used to assay OraSure HIV-1 oral fluid specimens obtained using the OraSure HIV-1 Oral Specimen Collection Device.

### A. Specimen Collection

1. Refer to the OraSure HIV-1 Oral Specimen Collection Device package insert for instructions on collecting a specimen.
2. OraSure HIV-1 specimens must be transported to the laboratory in the OraSure HIV-1 Specimen Vial.
3. OraSure HIV-1 specimens may be transported to the laboratory at ambient temperature via courier, air freight, or regular mail. OraSure specimens should be protected from impact, direct sunlight, and temperatures exceeding 37°C (98°F). Federal, state and local regulations regarding transportation of diagnostic specimens are applicable to OraSure HIV-1 specimens.

### B. Specimen Storage

1. After receipt at the laboratory, OraSure HIV-1 specimens (on or off the collection pad) should be stored at 2-8°C. Specimens may be stored at 4°C to 37°C for a maximum of 21 days from the time of collection, including the time for shipping and testing. If testing of specimens cannot be completed within 21 days, OraSure HIV-1 specimens can be stored frozen at -20°C for a maximum of six weeks.
2. OraSure HIV-1 specimens frozen and thawed once must be tested within the 21 days (see B.1 above). Specimens frozen and thawed twice must be tested within 24 hours, or discarded.

### C. Specimen Preparation

1. Record the specimen identification number from the OraSure HIV-1 Specimen Vial.
2. Ensure that the specimen is within acceptable dating for testing, i.e., <21 days from collection. Note: All testing should be completed within 21 days of specimen collection unless stored at -20°C (see B.1 above).
3. Hold the vial with the pointed tip up.
4. Move the pad away from the vial tip by gently tapping the vial.
5. Break the pointed tip of the vial off with the thumb.
6. Place a centrifuge tube over the vial and invert the tube and vial.
7. Centrifuge at 600-800 x g force for 15 minutes.
8. Determine that there is a minimum of 0.75 mL volume of specimen eluate.

If the volume of the centrifuged specimen is less than 0.75 mL, the specimen is unsuitable for testing and a new specimen from the test subject must be obtained. Notify the ordering physician if the volume of specimen is insufficient.

## PRELIMINARY PRECAUTIONS

1. Keep testing area separate from areas where 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 throughout the specimen processing and testing procedure.
5. Handle all materials used in the test (including specimens, Sample Buffer, reaction trays and pipets) as though capable of transmitting infectious agents.<sup>12</sup>
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 freshly prepared 1:10 dilution of  $\geq 5\%$  sodium hypochlorite (bleach) and dispose of the cleaning material by an appropriate method.
8. Dispose of all specimens and materials used to perform the test as if they contain infectious agents. Prior to disposal, treat as follows:

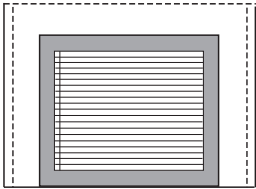
| Material         | Disposal Procedure  |
|------------------|---|
| Reusable items   | Autoclave for 60 minutes at 121°C.  |
| Disposable items | Incinerate.   |
| Liquid waste     | Mix with bleach to yield a final ratio of one part bleach to nine parts waste (1:10). Allow the mixture to stand 30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

## PROCEDURAL NOTES AND PRECAUTIONS

**Note:** This test kit may only be used to assay OraSure HIV-1 oral fluid specimens obtained using the OraSure HIV-1 Oral Specimen Collection Device. Do not test any specimens other than OraSure oral fluid specimens with this kit.

1. Do not interchange or combine any kit component, including strips, with components of another kit lot.
2. The OraSure Low Positive and Negative Controls must be assayed with **each** run. The OraSure High Positive must be assayed with the first run of every package of strips, but is optional in subsequent runs. This High Positive Control strip should be retained as a reference. It will be compared to the test strips run from that package to determine band identification and placement.
3. 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.
4. Prepare an assay worksheet, ensuring that the patient sample and control identification is linked to the number embossed on the nitrocellulose strip.
5. For sample dilution and addition to nitrocellulose strips, two options are offered (explained in detail on pages 8 and 9).
  - a. The first involves preparing the dilutions in test tubes and adding them to the strips.
  - b. The second involves adding the sample directly into the trough which contains the strip and Sample Buffer.



6. Avoid contamination of the strips and/or the buffer-soaked absorbent paper in the resealable pouch during handling (this may cause false reactivity in subsequent assays).
    - a. Prior to removing the strips from the pouch, clean the work surface and forceps with isopropyl alcohol.
    - b. Change gloves prior to opening the pouch.
    - c. Always use clean forceps when handling strips.
    - d. For initial use of strips, cut pouch below the seal line, keeping upper portion of pouch intact including seal line.
    - e. It is recommended that the lower portion of the strip pouch be cut on the remaining two sides (dotted lines as shown in diagram to the right).
    - f. Fold the plastic down, which acts as a protective barrier, to expose the strips.
- 
- The diagram shows a rectangular strip pouch. A solid grey border represents the top seal line. A dashed grey border represents the side and bottom cut lines. Inside the pouch, there are several horizontal lines representing the strips.
7. Place each prenumbered strip, with the green indicator line facing up, in the reaction trays in numerical order. This facilitates band alignment, for ease of reading results.
  8. Do not allow strips to dry out prior to sample addition. If diluting the samples in test tubes, place strips into trays only *after* dilutions have been made.
  9. As soon as the sample dilutions have been added to all strips in a tray, cover the tray with a lid.
  10. **It is essential to avoid cross-contamination between troughs, especially prior to and during sample incubation.**
    - a. Add sample to the trough of the reaction tray, using a transfer pipet for diluted samples or a pipet for undiluted samples.
    - b. It is suggested that an additional precaution be taken by positioning the strips in every other trough of a tray.
    - c. Avoid delivering bubbles to the liquid in the troughs.
    - d. Be careful to avoid dislodging fluid from the troughs when transferring trays.
    - e. Liquid in the troughs should not contact tray lids (if liquid should contact tray lids, immediately remove the material with a lab wipe).
  11. It is important that the items used to prepare and dispense Sample Buffer be scrupulously clean (a repeating pipet is preferable for dispensing the 1-2 mL of Sample Buffer).
  12. Prime the pipet tip when measuring samples or reagents.
  13. A rotation speed of 50-60 rpm is recommended for each rotation step.
  14. Be certain that each strip is immersed in the liquid and moves freely; however, liquid must not contact the tray lid during rotation.
  15. Incomplete or ineffective washing will compromise the assay; it is imperative to follow the wash procedure carefully.
  16. Discard used disposable reaction trays as biohazardous waste. Reuse of the trays and lids is not recommended.
  17. Samples must be at room temperature (20-25°C) before starting the test.

18. Reagents should be at room temperature (20-25°C) before beginning the assay except for Conjugate Concentrate and Substrate, which must both remain refrigerated (2-8°C) until just prior to use. Return all reagents to 2-8°C after use.

**CAUTION:** The Conjugate Concentrate and the buffer in the absorbent paper surrounding the strips contain sodium azide. If discarding into the sewer system, flush copiously with water. This helps prevent formation of metallic azides which, when highly concentrated in metal plumbing, may be potentially explosive. Decontaminate plumbing according to CDC guidelines.<sup>13</sup>

### ORASURE HIV-1 WESTERN BLOT TEST PROCEDURE

1. Prepare Sample Buffer as follows:
  - a. Check Sample Diluent Concentrate for crystals.
    - i. 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.
    - ii. 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* | mL of Deionized H <sub>2</sub> O required** | mL of Sample Diluent Conc. required** | g of Powdered Milk required*** |
|---------------------------------------|---------------------------------|---|---------------------------------------|--------------------------------|
| 3 - 6                                 | 200                             | 180   | 20                                    | 6                              |
| 7 - 9                                 | 300                             | 270   | 30                                    | 9                              |
| 10 - 13                               | 400                             | 360   | 40                                    | 12                             |
| 14 - 16                               | 500                             | 450   | 50                                    | 15                             |
| 17 - 20                               | 600                             | 540   | 60                                    | 18                             |

† Include strips for controls.

\* 30.0 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.

- c. Combine the required amounts of deionized water, Sample Diluent Concentrate and Powdered Milk.
  - d. Mix the solution for a minimum of 15 minutes (ensure the Powdered Milk is completely dissolved).
  - e. Store at room temperature while performing assay. Discard excess buffer at completion of assay.
2. Prepare an assay worksheet, ensuring that the patient sample and control identification is linked to the number embossed on the nitrocellulose strip.
3. Both the OraSure Low Positive and Negative Controls must be assayed with **each** run. The OraSure High Positive must be assayed with the first run of every package of strips, but is optional in subsequent runs. This High Positive Control strip should be retained as a reference. It will be compared to the test strips run from that package to determine band identification and placement.
4. Add controls and patient specimens to nitrocellulose strips by one of the following methods:

a. **METHOD I: Test Tube Dilution**

- i. Into appropriately labeled test tubes, add 150  $\mu\text{L}$  of each specimen or control to 1.0 mL of Sample Buffer and mix well. These dilutions must be tested within an hour.
- ii. Place one prenumbered strip with *green indicator line facing up* into each trough as follows, ensuring the strips do not dry out:
  - a) For initial use of strips, cut pouch below the seal line, keeping upper portion of pouch intact including seal line.
  - b) Cut the lower portion of the pouch on the two remaining sides (see page 7, step 6.e).
  - c) Fold the packaging back to expose strips.
  - d) Beginning with the left side of the series (strip #1), remove strips to be assayed and place them in numerical order.
  - e) Grasp the strip at the green indicator line with forceps.
  - f) Transfer the strips, avoiding contact with contaminated surfaces, into the troughs of the reaction tray(s).
  - g) Place any remaining strips (still encased in moist blotting paper and contained in the lower portion of the pouch) in the upper portion of pouch, seal using zip closure, and return to storage at 2-8°C.
- iii. Transfer the contents of each tube (~1.1 mL) into the corresponding trough using a transfer pipet.

b. **METHOD II: On-Strip Dilution**

- i. Add 1.0 mL Sample Buffer to each trough to be used.
  - ii. Place one prenumbered strip with *green indicator line facing up* into each trough as follows, ensuring the strips do not dry out:
    - a) For initial use of strips, cut pouch below the seal line, keeping upper portion of pouch intact including seal line.
    - b) Cut the lower portion of the pouch on the two remaining sides (see page 7, step 6.e).
    - c) Fold the packaging back to expose strips.
    - d) Beginning with the left side of the series (strip #1), remove strips to be assayed and place them in numerical order.
    - e) Grasp the strip at the green indicator line with forceps.
    - f) Transfer the strips, avoiding contact with contaminated surfaces, into the troughs of the reaction tray(s).
    - g) Place any remaining strips (still encased in moist blotting paper and contained in the lower portion of the pouch) in the upper portion of pouch, seal using zip closure, and return to storage at 2-8°C.
  - iii. Add 150  $\mu\text{L}$  of each OraSure specimen or control to the corresponding trough.
5. Cover each tray with a lid and mix by gentle rotation (50-60 rpm) on a rotator for 180 minutes (3 hours) at room temperature.
  6. After incubation, completely aspirate the liquid from troughs (do not allow the strips to dry).

7. Wash the strips as follows:
  - a. Add 2.0 mL Sample Buffer to each strip.
  - b. Immediately aspirate all liquid from each trough.
  - c. Repeat steps a and b two more times.
  - d. Add 2.0 mL Sample Buffer to each strip and replace the lid(s).
  - e. Place the tray(s) on the rotator (at 50-60 rpm) for 5 minutes.
  - f. Aspirate the Sample Buffer completely.
  - g. Repeat steps d and e one more time.
8. Prepare diluted Conjugate during the final wash step (step 7.g above):
  - a. Conjugate dilution must be prepared during the last five minute wash and applied immediately after the wash is complete and the wash solution is aspirated. Excess Conjugate dilution must be discarded.
  - b. Remove Conjugate Concentrate from the refrigerator.
  - 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 Concentrate required** | Total number of strips being assayed† | mL of Sample Buffer required* | µL of Conjugate Concentrate required** |
|---------------------------------------|-------------------------------|--|---------------------------------------|-------------------------------|--|
| 3                                     | 8                             | 40                                     | 12                                    | 26                            | 130                                    |
| 4                                     | 10                            | 50                                     | 13                                    | 28                            | 140                                    |
| 5                                     | 12                            | 60                                     | 14                                    | 30                            | 150                                    |
| 6                                     | 14                            | 70                                     | 15                                    | 32                            | 160                                    |
| 7                                     | 16                            | 80                                     | 16                                    | 34                            | 170                                    |
| 8                                     | 18                            | 90                                     | 17                                    | 36                            | 180                                    |
| 9                                     | 20                            | 100                                    | 18                                    | 38                            | 190                                    |
| 10                                    | 22                            | 110                                    | 19                                    | 40                            | 200                                    |
| 11                                    | 24                            | 120                                    | 20                                    | 42                            | 210                                    |

† 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 1:201.

- 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.
9. Aspirate the Sample Buffer from the troughs and add 2.0 mL diluted Conjugate to each strip.
10. Replace each lid and incubate on the rotator (at 50-60 rpm) for 45 minutes at room temperature.
11. Repeat wash procedure from steps 6 and 7.
12. Completely aspirate the Sample Buffer and add 2.0 mL of deionized water to each strip.
13. Replace each lid and place on rotator (at 50-60 rpm) for 5 minutes.
14. Remove Substrate from refrigerator during final wash (step 13).
15. Aspirate deionized water from each strip.
16. Add 1.0 mL of Substrate to each strip.

17. Replace each lid and *gently* move trays back and forth 2-3 times by hand on work surface to ensure strips are completely immersed in Substrate.
18. Incubate for exactly 10 minutes at room temperature without rotation. **Note:** It is important not to exceed the 10 minute Substrate incubation time.
19. Stop the color development of the strips as follows:
  - a. Aspirate the Substrate.
  - b. Add 2.0 mL of deionized water to each strip.
  - c. Immediately aspirate the contents of the tray(s).
  - d. Repeat steps b and c two more times.
  - e. Add 2.0 mL deionized water to each strip and replace the tray lid(s).
  - f. Place on rotator for 5 minutes.
  - g. Aspirate water completely.
20. Allow the developed strips to air dry in the tray(s).
21. Handle the 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 in the dark at room temperature.

## QUALITY CONTROL

Both the OraSure Low Positive and Negative Controls must be assayed regardless of the number of samples tested. The OraSure High Positive must be assayed with the first run of every package of strips, but is optional in subsequent runs. This High Positive Control strip should be retained 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 the assay to be considered valid:

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, page 13).  
(*Strongly Reactive*)

## INTERPRETATION OF RESULTS

1. Band Identification
  - a. Correlate the band position of the OraSure High Positive Control strip with Figure 1 on page 13 to identify the HIV-1 viral bands and their positions.
  - b. Compare each test strip to the OraSure High Positive Control strip for identification of reactive bands.
2. Band Intensity
  - a. Compare the bands of each strip and control to the gp41 band on the OraSure Low Positive Control strip and assign a level of intensity as follows:

| Assignment        | Definition  |
|-------------------|---|
| Present (P)       | The band intensity is greater than or equal to the gp41 band on the OraSure Low Positive Control strip. |
| Indeterminate (I) | The band is visible but intensity is less than the gp41 band on the OraSure Low Positive Control strip. |
| Absent (A)        | No reactivity is observed.  |

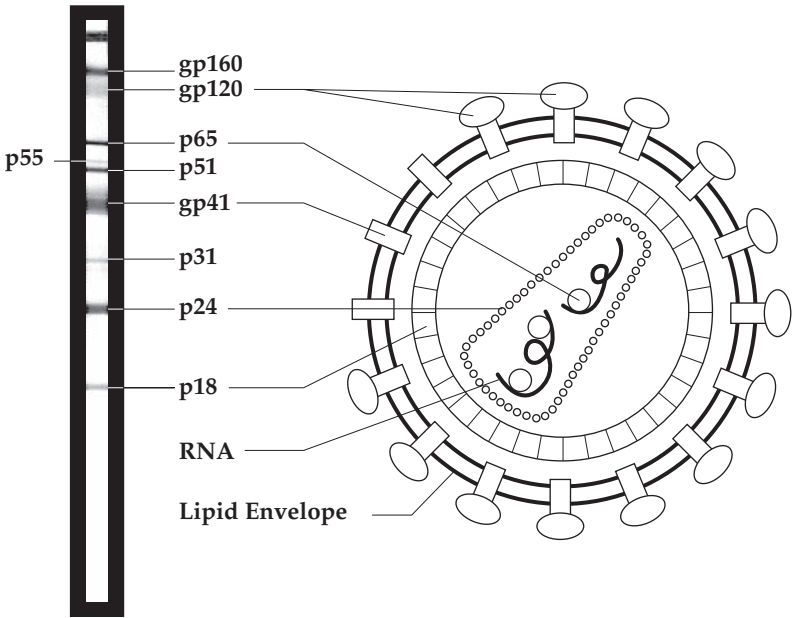
3. Strip Interpretation
  - a. Based on band position and reactivity, analyze the results and assign each strip a final result.

| Test Result   | Definition  |
|---------------|---|
| Positive      | <u>Any two</u> of the three major bands of diagnostic significance below must be <i>Present</i> .<br>gp160 and/or gp41 p24<br>gp120<br>Other bands may or may not be present. |
| Indeterminate | <u>Any</u> visible 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 OraSure® HIV-1 Western Blot Strip**

On the left is a representation of an OraSure Western blot strip developed with OraSure High Positive Control. The illustration is a reference for band identification and position (see Interpretation of Results, page 12, step 1).

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



Actual Size

Figure 1. HIV-1 Virion

**Viral Origin of HIV-1 Associated Bands**

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

## LIMITATIONS OF THE PROCEDURE

1. The assay must be performed in strict accordance with these instructions to obtain accurate, reproducible results.
2. 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.
3. Individuals may present incomplete banding patterns due to the natural history of AIDS or other immunodeficiency states, e.g.:
  - a. AIDS patients may lose antibody reactions to p24 and p31;
  - b. Infants born to HIV-1 infected mothers, but who are uninfected may display incomplete patterns as passively acquired maternal antibodies begin to disappear;
  - c. Individuals who have recently seroconverted may display incomplete band patterns;
  - d. Infected patients with malignancies and individuals receiving immunosuppressive drugs may fail to develop a Positive result;
  - e. Individuals infected with HTLV-I/II or HIV-2, may exhibit cross-reactivity;
  - f. Individuals may develop incomplete patterns that reflect the composition of experimental HIV sub-unit vaccines that they may have received.
4. 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.
5. Since reactivity of any degree with any of the 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>
6. Do not use this kit as the sole basis of diagnosis of HIV-1 infection.
7. A Negative result does not exclude the possibility of HIV-1 infection.
8. The OraSure HIV-1 Western Blot Kit is a biological product which, although highly consistent, does display variation from lot to lot. Examples of these variations include bands which have a 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 the assay interpretation, call the assay invalid and repeat.

## PERFORMANCE CHARACTERISTICS

The performance of the OraSure HIV-1 Western Blot Kit was evaluated by comparison of OraSure results with those obtained from matched serum specimens tested by a licensed HIV-1 Western blot. These specimens were collected prospectively in a clinical study of low risk (n = 2,382), high risk (n = 698), and AIDS (n = 242) populations. In addition, non-specificity specimens (n = 248) were obtained from subjects with non-HIV-1 related medical conditions that might result in antibodies cross-reactive with HIV-1 proteins. All of the high risk and AIDS subjects, and 495 of the low risk subjects were tested by Western blot irrespective of their EIA results. EIA testing of an additional 1,887 "screen only" low risk subjects was carried out using the bioMérieux Oral Fluid Vironostika HIV-1 Microelisa System in an effort to find EIA repeatedly reactive samples (from uninfected individuals) with which to challenge the OraSure Western blot. Testing of the 1,887 "screen only" subjects identified 14 OraSure specimens as repeatedly reactive.



These 14 OraSure specimens and their matching sera were also advanced to Western blot testing. Thus, a total of 1,697 matched OraSure and serum specimens were tested by Western blot at five testing laboratories throughout the United States.

Low risk subjects, primarily normal blood donors, were persons with no known risk factors. Of the 698 high risk specimens, 363 were from homosexuals, 116 from injection drug users (IDUs), 83 from persons with multiple heterosexual contacts, and 44 from hemophiliacs. The remaining 92 high risk subjects included bisexuals, prostitutes, and individuals with other acknowledged risk factors. Specimens from 242 persons with clinically diagnosed AIDS were also tested.

The frequency of virus-specific bands and interpretation by risk group using the OraSure HIV-1 Western Blot Kit are presented in Table 1.

**Table 1. Frequency of Virus-Specific Bands (“Present” or “Indeterminate”) and Interpretation of Specimens Tested by the OraSure HIV-1 Western Blot Kit**

| Low Risk <sup>a</sup>                     | OraSure HIV-1 WB Result        | Band Specificities (# and % of samples) <sup>g</sup> |               |               |               |               |               |               |               |               | Non-Viral    |            |
|---|--------------------------------|--|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|--------------|------------|
|   |                                | gp160  | gp120         | p65           | p55           | p51           | gp41          | p31           | p24           | p18           |              |            |
| EIA neg.<br>n=495                         | POS 0<br>(0.0)                 | 0<br>(0.0)   | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)   | 0<br>(0.0) |
|   | IND 100 <sup>b</sup><br>(20.2) | 0<br>(0.0)   | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 5<br>(1.0)    | 0<br>(0.0)    | 13<br>(2.6)   | 2<br>(0.4)    | 87<br>(17.6) |            |
| EIA RR <sup>h</sup><br>n=14               | POS 0<br>(0.0)                 | 0<br>(0.0)   | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)   | 0<br>(0.0) |
|   | IND 3 <sup>c</sup><br>(21.4)   | 0<br>(0.0)   | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 1<br>(7.1)    | 0<br>(0.0)    | 2<br>(14.3)  |            |
| High Risk <sup>d</sup>                    | OraSure HIV-1 WB Result        | Band Specificities (# and % of samples) <sup>g</sup> |               |               |               |               |               |               |               |               | Non-Viral    |            |
|   |                                | gp160  | gp120         | p65           | p55           | p51           | gp41          | p31           | p24           | p18           |              |            |
| EIA neg.<br>n=264                         | POS 0<br>(0.0)                 | 0<br>(0.0)   | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)   | 0<br>(0.0) |
|   | IND 45 <sup>e</sup><br>(17.0)  | 4<br>(1.5)   | 0<br>(0.0)    | 6<br>(2.3)    | 0<br>(0.0)    | 5<br>(1.9)    | 0<br>(0.0)    | 0<br>(0.0)    | 8<br>(3.0)    | 0<br>(0.0)    | 33<br>(12.5) |            |
| EIA RR <sup>h</sup><br>n=434 <sup>i</sup> | POS 429<br>(98.8)              | 429<br>(98.8)  | 427<br>(98.4) | 417<br>(96.1) | 160<br>(36.9) | 412<br>(94.9) | 427<br>(98.4) | 394<br>(90.8) | 410<br>(94.5) | 238<br>(54.8) | 1<br>(0.2)   |            |
|   | IND 3<br>(0.7)                 | 3<br>(0.7)   | 2<br>(0.5)    | 2<br>(0.5)    | 0<br>(0.0)    | 2<br>(0.5)    | 1<br>(0.2)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 1<br>(0.2)   |            |
| AIDS <sup>f</sup>                         | OraSure HIV-1 WB Result        | Band Specificities (# and % of samples) <sup>g</sup> |               |               |               |               |               |               |               |               | Non-Viral    |            |
|   |                                | gp160  | gp120         | p65           | p55           | p51           | gp41          | p31           | p24           | p18           |              |            |
| EIA neg.<br>n=1                           | POS 0<br>(0.0)                 | 0<br>(0.0)   | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)   | 0<br>(0.0) |
|   | IND 1<br>(100.0)               | 1<br>(100.0)   | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 0<br>(0.0)    | 1<br>(100.0)  | 0<br>(0.0)    | 0<br>(0.0)   |            |
| EIA RR <sup>h</sup><br>n=241              | POS 236<br>(97.9)              | 236<br>(97.9)  | 236<br>(97.9) | 211<br>(87.6) | 35<br>(14.5)  | 207<br>(85.9) | 236<br>(97.9) | 190<br>(78.8) | 189<br>(78.4) | 87<br>(36.1)  | 0<br>(0.0)   |            |
|   | IND 5<br>(2.1)                 | 5<br>(2.1)   | 4<br>(1.7)    | 1<br>(0.4)    | 0<br>(0.0)    | 1<br>(0.4)    | 4<br>(1.7)    | 1<br>(0.4)    | 0<br>(0.0)    | 1<br>(0.4)    | 0<br>(0.0)   |            |

a. Persons with no known risk factors; primarily normal blood donors.

b. 80 of 100 results Indeterminate due to non-viral bands only.

c. 2 of 3 results Indeterminate due to non-viral bands only.

d. Homosexuals, IDUs, and other accepted risk designations.

e. 32 of 45 results Indeterminate due to non-viral bands only.

f. CDC Classification; MMWR 1982; 31: 507-508.

g. Band patterns for negative samples do not appear in this table. By definition, negative samples show no reactivity.

h. RR indicates repeatedly reactive OraSure EIA results.

i. Includes two EIA false positive individuals whose OraSure Western blots were negative.

## Sensitivity Studies

The performance of the OraSure Western blot in seropositive subjects was evaluated by comparing results to those obtained by testing matched serum samples collected from individuals at high risk for HIV infection and from clinically diagnosed AIDS patients. A comparison of OraSure and serum results is presented in Table 2.

**Table 2. OraSure and Serum Western Blot Results for Confirmed Positive High Risk and AIDS Populations**

| Risk Category | Confirmed Positives | OraSure Specimen Results |              |                  |   | Serum Results |   |   |
|---------------|---------------------|--------------------------|--------------|------------------|---|---------------|---|---|
|               |                     | EIA                      | Western Blot |                  |   | Western Blot  |   |   |
|               |                     | RR                       | P            | I                | N | P             | I | N |
| AIDS          | 242                 | 241 <sup>a</sup>         | 236          | 6 <sup>a,b</sup> | 0 | 241           | 1 | 0 |
| High Risk     | 431                 | 431                      | 429          | 2 <sup>c</sup>   | 0 | 431           | 0 | 0 |
| Total         | 673                 | 672                      | 665          | 8                | 0 | 672           | 1 | 0 |

a. One OraSure sample was EIA false negative, Western blot indeterminate (gp160+, p24±); the matching serum sample was EIA repeatedly reactive and Western blot positive (gp160+, gp120+, p65+, p51+, p24+).

b. One of the six Western blot indeterminate OraSure samples was repeatedly reactive on EIA and concordant on Western blot with an indeterminate result for the matching serum specimen. The remaining five OraSure specimens were discordant (indeterminate) with the matching serum specimens (positive) due to the required cardinal bands being visible but of insufficient intensity to be scored as Present.

c. One OraSure sample was discordant due to the intensity of bands on the OraSure Western blot (gp160+, gp120±, p65±, p51±, gp41±). The banding pattern of the second indeterminate OraSure sample was gp160+, gp120±. The matching serum specimens' banding patterns were gp160+, gp120+, p65+, p51±, gp41+, p24±, p18±, and gp160+, gp120+, p65±, gp41+, p24±, p18±, respectively.

RR = Repeatedly Reactive; P = Positive; I = Indeterminate; N = Negative.

In this study, the sensitivity of the OraSure Western blot testing of oral specimens from the 242 confirmed positive AIDS subjects was 97.5% (236/242) with 2.5% (6/242) indeterminate, and from the 431 confirmed high risk subjects was 99.5% (429/431) with 0.5% (2/431) indeterminate, with no OraSure Western blot false negatives in either group. All OraSure indeterminate blots showed the gp160 band as present and at least one additional cardinal band (gp120, gp41, p24) as visible, but of insufficient intensity to be called present. One of the OraSure indeterminate blots corresponded to the Western blot indeterminate serum specimen.

## Specificity Studies

The performance of the OraSure Western blot in documented seronegative subjects was evaluated by testing specimens from 495 EIA negative subjects (using oral fluid) at low risk for HIV-1 infection, 14 EIA repeatedly reactive specimens found by screening 1,887 persons at low risk for HIV-1 infection, 248 subjects with non-HIV related medical conditions (non-specificity subjects), and 267 specimens from high risk seronegative subjects. Thus, a total of 1,024 OraSure HIV-1 Western blots and serum Western blots were performed on these individuals. The results of this testing are presented in Table 3.

**Table 3. Comparative Study of Western Blot Results in Low Risk, Non-Specificity, and High Risk, HIV-1 Negative Populations**

| EIA Result for OraSure Specimens | OraSure HIV-1 Western Blot Interpretation | Licensed Serum HIV-1 Western Blot Interpretation |      |          | Totals |
|----------------------------------|---|--|------|----------|--------|
|                                  |   | Positive   | Ind. | Negative |        |
| EIA Negative (n=1,007)           | Positive                                  | 0  | 0    | 0        | 0      |
|                                  | Indeterminate                             | 0  | 96   | 111      | 207    |
|                                  | Negative                                  | 0  | 295  | 505      | 800    |
| EIA RR (n=17)                    | Positive                                  | 0  | 0    | 0        | 0      |
|                                  | Indeterminate                             | 0  | 3    | 1        | 4      |
|                                  | Negative                                  | 0  | 7    | 6        | 13     |
| Totals                           |   | 0  | 401  | 623      | 1,024  |

Seventeen OraSure specimens (14 low risk and 3 high risk) were EIA repeatedly reactive. Thirteen of the 17 EIA false positive specimens were correctly identified as negative by the OraSure HIV-1 Western blot. Thus 2,893 out of 2,897 subjects (99.9%) were correctly identified as HIV-1 antibody negative by a combination of EIA and Western blot testing of OraSure samples. The four remaining specimens were indeterminate by OraSure HIV-1 Western blot (two of the four due to non-viral bands only). The indeterminate rate for uninfected persons who are EIA repeatedly reactive by OraSure was 23.5% (4/17) as compared to 58.8% (10/17) for serum.

Western blot was also performed on serum and OraSure specimens from 1,007 EIA negative subjects (using oral fluid). This testing identified 20.6% of OraSure specimens and 39.2% of sera as indeterminate. The overall concordance between the two types of specimens was 59.6%. Differences were largely due to non-viral bands that were present for one type of sample but not the other for individual subjects.

## **Analytical Sensitivity**

### ***Titration of Matching OraSure and Serum Specimens***

Fifteen randomly selected matching OraSure and serum repository specimens that had been obtained from HIV-positive individuals were titrated. Titrated serum specimens were tested with the licensed serum HIV-1 Western Blot Kit and matching titrated OraSure specimens were tested in parallel with the OraSure HIV-1 Western Blot Kit. The assay endpoint in this study was the last dilution at which a positive Western blot result was observed for each specimen. The results of this study are shown in Table 4.

Endpoints were obtained for all serum specimens and for 14 of the 15 OraSure specimens tested. The one specimen not yielding an endpoint (ID# 19052) had an indeterminate result when the neat OraSure specimen was tested. This assignment was based on positive reactivity for the gp160 and gp120 bands, and an indeterminate reactivity for the gp41 band. The corresponding serum specimen for subject 19052 also had a comparatively low titer (1:4). A review of medical records revealed that this individual was severely immunocompromised at the time of specimen acquisition (CD4<sup>+</sup> count = 18/mm<sup>3</sup>).

The average ratio of the serum endpoints to the OraSure endpoints was 5.7:1. The difference in analytical sensitivity between the licensed serum Western Blot Kit and the OraSure Western Blot Kit ranged from a ratio of 12.8 to 0.40.

**Table 4. Highest Dilution Yielding Positive Western Blot Results for 15 Matching OraSure and Serum Specimens**

| Specimen Number   | Highest OraSure Dilution <sup>a</sup> | Highest Serum Dilution <sup>a</sup> | Ratio <sup>b</sup> |
|-------------------|---------------------------------------|-------------------------------------|--------------------|
| 19026             | 1:20                                  | 1:256                               | 12.8               |
| 19027             | 1:20                                  | 1:256                               | 12.8               |
| 19078             | 1:20                                  | 1:256                               | 12.8               |
| 19095             | 1:20                                  | 1:256                               | 12.8               |
| 19105             | 1:5                                   | 1:64                                | 12.8               |
| 19079             | 1:10                                  | 1:64                                | 6.4                |
| 19032             | 1:10                                  | 1:16                                | 1.6                |
| 19059             | 1:10                                  | 1:16                                | 1.6                |
| 19066             | 1:50                                  | 1:64                                | 1.28               |
| 19069             | 1:50                                  | 1:64                                | 1.28               |
| 19080             | 1:250                                 | 1:256                               | 1.02               |
| 19044             | 1:5                                   | 1:4                                 | 0.8                |
| 19101             | 1:100                                 | 1:64                                | 0.64               |
| 19033             | 1:10                                  | 1:4                                 | 0.40               |
| <b>Mean ratio</b> |                                       |                                     | 5.7                |
| 19052             | Neat <sup>c</sup>                     | 1:4                                 | -                  |

a. Beyond standard specimen dilution per assay protocol.

b. Ratio of serum endpoint dilution/OraSure endpoint dilution.

c. Specimen #19052 was indeterminate (with viral bands) when the undiluted OraSure specimen was tested.

### ***Titration of OraSure Seroconversion Specimens***

Repository OraSure and plasma specimens from an earlier seroconversion study were used to assess the analytical sensitivity of the OraSure HIV-1 Western Blot.

OraSure specimens were diluted and each dilution was assayed by EIA and Western blot. The objective of this study was to determine the highest dilution of the OraSure specimen that would produce a repeatedly reactive EIA result, based on product insert criteria, and would demonstrate viral band reactivity in the OraSure HIV-1 Western Blot Kit.

Table 5 shows the results of this testing. For each of the four time points, the OraSure Western Blot demonstrated viral bands at dilutions which produced non-reactive EIA results, yielding an average of  $\geq 10$ -fold enhanced sensitivity over the EIA for OraSure specimens.

**Table 5. Reactivity of OraSure HIV-1 Seroconversion Specimens in the Oral Fluid Vironostika HIV-1 Microelisa System and the OraSure HIV-1 Western Blot Kit**

| ID#        | Highest EIA Dilution | Highest WB Dilution | Blot to EIA Sensitivity |
|------------|----------------------|---------------------|-------------------------|
| 01SC052591 | Negative at Neat     | Positive at 1:10*   | $\geq 10$               |
| 01SC052691 | RR at Neat           | Positive at 1:5*    | $\geq 5$                |
| 01SC052791 | RR at Neat           | Positive at 1:5*    | $\geq 5$                |
| 01SC052991 | RR at Neat           | Positive at 1:20*   | $\geq 20$               |

\* Indicates the highest dilution tested RR = Repeatedly Reactive

### **Reproducibility**

The reproducibility of the OraSure HIV-1 Western Blot Kit was evaluated at three separate test laboratories. The study included testing a three-member panel of pooled OraSure specimens with the OraSure HIV-1 Western Blot Kit. The OraSure reproducibility panel consisted of an HIV-1 antibody positive specimen, an HIV-1 antibody negative specimen, and an HIV-1 Western blot indeterminate specimen.

The panel members were tested on three separate days, using three separate OraSure HIV-1 Western Blot Kit production lots, resulting in a total of 27 test results being generated for each panel member. The percentage of times each band was scored reactive is presented in Table 6.

**Table 6. Reproducibility of the OraSure HIV-1 Western Blot Kit**

| Specimen # | Specimen Reactivity        | Percent Frequency of Visible Bands <sup>c</sup> |       |     |     |     |      |     |      |      |
|------------|----------------------------|---|-------|-----|-----|-----|------|-----|------|------|
|            |                            | gp160   | gp120 | p65 | p55 | p51 | gp41 | p31 | p24  | p18  |
| 1          | Positive <sup>a</sup>      | 100   | 100   | 100 | 0   | 100 | 100  | 100 | 100  | 18.5 |
| 2          | Negative                   | 0   | 0     | 0   | 0   | 0   | 0    | 0   | 0    | 0    |
| 3          | Indeterminate <sup>b</sup> | 100   | 100   | 100 | 0   | 100 | 100  | 3.7 | 70.4 | 0    |

a. Positive specimen known banding pattern: reactivity for gp160, gp120, p65, p51, gp41, p31, p24 and no reactivity for p55, p18.

b. Indeterminate specimen known banding pattern: reactivity for gp160, gp120, p65, p51, gp41, p24; and no reactivity for p55, p31, p18.

c. Frequency of visible bands (either Indeterminate or Present).

The results demonstrate that for positive specimens, negative specimens, and indeterminate specimens with known banding patterns, reproducibility is high.

### Reactivity in Other Disease Conditions

Matching OraSure and serum specimens were obtained at three sites from 248 subjects who were enrolled in the clinical trial because they had non-HIV-1 medical conditions that might result in antibodies cross-reactive with HIV-1 proteins or other potentially interfering factors. Specimens studied included 89 from multiparous women, 69 from subjects with non-HIV viral infections, 50 receiving anticoagulation therapy, 26 with autoimmune diseases other than AIDS, 11 with oral pathology, and 3 with polyclonal or monoclonal gammopathy. Although bands were present at viral band locations for four samples (1.6%), none of the strips could be interpreted as positive. Results are presented in Table 7.

**Table 7. Results of OraSure Western Blot Testing on Samples from Subjects with Non-HIV Disease Processes or Other Potentially Interfering Factors**

| OraSure HIV-1 WB Result       | Band Specificities (# and % of samples) |            |            |            |            |            |            |            |            | Non-viral    |
|-------------------------------|---|------------|------------|------------|------------|------------|------------|------------|------------|--------------|
|                               | gp160                                   | gp120      | p65        | p55        | p51        | gp41       | p31        | p24        | p18        |              |
| NEG 186<br>(75.0)             | 0<br>(0.0)                              | 0<br>(0.0) | 0<br>(0.0) | 0<br>(0.0) | 0<br>(0.0) | 0<br>(0.0) | 0<br>(0.0) | 0<br>(0.0) | 0<br>(0.0) | 0<br>(0.0)   |
| IND 62 <sup>a</sup><br>(25.0) | 0<br>(0.0)                              | 0<br>(0.0) | 0<br>(0.0) | 0<br>(0.0) | 0<br>(0.0) | 2<br>(3.2) | 0<br>(0.0) | 2<br>(3.2) | 1<br>(1.6) | 58<br>(93.5) |
| Total 248<br>(100.0)          | 0<br>(0.0)                              | 0<br>(0.0) | 0<br>(0.0) | 0<br>(0.0) | 0<br>(0.0) | 2<br>(0.8) | 0<br>(0.0) | 2<br>(0.8) | 1<br>(0.4) | 58<br>(23.4) |

a. 58 of 62 results Indeterminate due to non-viral bands only.

Testing of specimens from this population revealed that the number of OraSure indeterminates (62; 25.0%) was substantially less than the number of serum indeterminates (121; 48.8%). The number of serum indeterminates due to the presence of viral bands (27; 10.9%) was substantially greater than the number of OraSure indeterminates due to the presence of viral bands (4; 1.6%).

## Summary

In this clinical trial using the recommended OraSure algorithm, 3,558/3,570 subjects received the correct HIV-1 antibody results from a single OraSure sample the first time it was tested. In 11 of the remaining subjects, the Western blot was indeterminate: for these 11, the algorithm would lead to appropriate follow-up testing. Thus, in 3,569/3,570 (99.97%) subjects either the correct result was reached or appropriate follow-up testing would be triggered. It is concluded that OraSure testing is a highly accurate alternative to serum testing.

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