Avioq HIV-1 Microelisa System

Store between 2-8°C.
For *in vitro* diagnostic use.

INTENDED USE

The Avioq HIV-1 Microelisa System is an enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) in human specimens collected as serum, plasma, dried blood spots, or oral fluid specimens obtained with OraSure®.HIV-1 Oral Specimen Collection Device. The Avioq HIV-1 Microelisa System is intended for use as an aid in diagnosis of infection with HIV-1. It is not intended for use in screening blood donors.

Note: See Warnings, Interpretation of results, Limitations and Performance characteristics sections for information on:

1. Reduced sensitivity and specificity of testing with OraSure® HIV-1 specimens compared with testing blood specimens.
2. The need for follow up testing with a blood specimen when subjects have repeatedly reactive ELISA results using OraSure® HIV-1 specimens.
3. Reporting test results to the ordering physician or someone under the supervision of the ordering physician.

SUMMARY AND EXPLANATION OF THE TEST

Published data indicate a strong correlation between the acquired immunodeficiency syndrome (AIDS) and a retrovirus referred to as Human Immunodeficiency Virus (HIV).[1,2] Currently, two HIV serotypes, designated as HIV-1 and HIV-2, have been identified based on the results of serologic and molecular studies. Both HIV serotypes have been isolated from patients with AIDS and AIDS-related complex (ARC), as well as from apparently healthy individuals at high risk for AIDS.[2] Both viruses have the same morphology, lymphotropism,[3] and modes of transmission.[4] Since 1984, reports have indicated that HIV-1 can be isolated from a variety of tissues and body fluids of infected individuals.[2,5]

Following infection with HIV, an individual rapidly (*e.g*. within 4 weeks) develops antibodies to viral proteins, a process known as seroconversion. After seroconversion, HIV specific antibodies can be readily detected in the blood specimen. A majority of patients who exhibit symptoms of AIDS or ARC have HIV specific antibodies in their blood. In addition, a significant proportion of apparently healthy individuals at increased risk for AIDS also contain HIV specific antibodies in their blood specimens. Due to close relation of HIV-1 and HIV-2, proteins of the two viruses, especially the core and polymerase proteins, result in serologic cross-reactivity[3], particularly when HIV viral lysate, which contains all viral proteins, is used as the antigens for antibody detection.

It is well established that HIV virus undergoes pronounced mutations, particularly under selection pressures. Consequently, an increasing number of variants have been identified with HIV-1, some of which have arisen from genetic recombinations within a host having infection with multiple types of HIV-1. Such variants may be more difficult to be detected with ELISA systems that are not based on viral lysate.[6,7] Worldwide distribution of HIV shows prevalence of the serotypes in different areas, with HIV-1 most widely distributed.

The Avioq HIV-1 Microelisa System assay was designed to be highly sensitive for a spectrum of HIV-1 serotypes. As a result, nonspecific reactions may occasionally be seen in specimens from people who have prior pregnancy, blood transfusion, or exposure to human cells or media containing cultured HIV antigen.[8] Because of these and other potential nonspecific reactions, specimens reactive with the Avioq HIV-1 Microelisa System assay should be analyzed with a confirmatory test, *e.g*., Western Blot test.

Reactive specimens upon initial testing with the Avioq HIV-1 Microelisa System assay should be re-tested in duplicate. Reactivity in either or both of the duplicate tests indicates a potential for the presence of HIV-specific antibody. In individuals at increased risk of infection, such as homosexual men, hemophiliacs, or

intravenous drug users, repeatedly reactive specimens are usually found to contain antibodies to HIV by additional, more specific tests. However, when the ELISA is used to screen populations with a low prevalence of HIV infections, nonspecific reactions may be more common than specific reactions. Information about prevalence of HIV infections in persons in various categories of risk, as well as clinical and public health guidelines, are available in each CDC publication of *Morbidity and Mortality Weekly Reports*. Although information about the degree of risk for HIV-1 infection and the degree of reactivity of the serum are of value in interpreting the test, a diagnosis should not be based only on this information. Therefore, it is appropriate to investigate repeatedly reactive specimens by additional, more specific tests, such as Western Blot, immunofluorescence, radioimmunoprecipitation, viral antigen based immunoassays, peptide ELISAs, or nucleic acid amplification assays.

PRINCIPLE OF THE TEST

This test uses HIV-1 antigens, including inactivated, purified HIV-1 viral lysate proteins[9], which are coated onto the wells of microwell plates, for the detection of antibodies against HIV-1. Upon addition of a diluted test specimen containing antibodies to HIV-1 to a microwell, immune complexes are formed through the interaction between anti-HIV in the specimen and HIV antigens coated on the microwell. Following incubation, the specimen is aspirated and the well is washed with buffer. Subsequently, anti-human immunoglobulin (goat) conjugated with horseradish peroxidase (HRP) is added which binds to the anti-HIV-antigen complex during a second incubation. Following a wash and incubation with ABTS (2,2'-azino-di-[3-ethylbenzthiazoline-6-sulfonate]) substrate, a green color is produced. The enzyme reaction is stopped by the addition of a fluoride solution.[10] The amounts of antibodies to HIV present in the specimen are qualitatively proportional to color intensity. Because of the use of HIV-1 viral lysate as the primary antigens in the test, significant cross reactivity with HIV-2 specimens was observed.

REAGENTS

For *in vitro* Diagnostic Use.

Components in each Avioq HIV-1 Microelisa System

384 Tests	960 Tests	9,600 Tests	Component Description
4 stripholders	10 stripholders	100 stripholders	**HIV-1 Microelisa Strips** – Twelve per holder, each containing HIV-1 antigen coated wells, including inactivated, purified viral lysate protein, contained in a foil pack (2 stripholders/pack) with silica gel desiccant.
1 bottle (120 ml)	2 bottles (120 ml)	20 bottles (120 ml)	**Dilsim™ III** – Liquid specimen diluent; contains bovine and caprine proteins, salt, surfactant, Chlorophenol Red as specimen addition indicator, and antimicrobial agents (contains 0.03% [w/v] bromonitrodioxane).
3 vials (0.5 ml)	6 vials (0.5 ml)	18 vials (0.5 ml)	**Negative Calibrator Serum (Human)** – Contains human serum and goat serum as stabilizer with 0.05% (w/v) bromonitrodioxane as preservative and Fast Green FCF as coloring agent; no detectable antibodies to HBsAg, HIV-1 or HCV.
1 vial (0.5 ml)	2 vials (0.5 ml)	6 vials (0.5 ml)	**HIV-1 Positive Control Serum (Human)** – Inactivated human serum containing protein stabilizers. Contains 0.05% (w/v) bromonitrodioxane as preservative and coloring agent; reactive for antibodies to HIV-1 and nonreactive to HBsAg and antibodies to HCV.

384 Tests	960 Tests	9,600 Tests	Component Description
2 vials	4 vials	38 vials	**Peroxidase Conjugated Goat Anti-human Immunoglobulins (EnzAbody®)** – Lyophilized with protein stabilizers and FD&C red dye no. 2.
1 bottle (120 ml)	2 bottles (120 ml)	16 bottles (120 ml)	**Conjugate Diluent** – Phosphate buffered saline containing protein stabilizers and 0.03% (w/v) bromonitrodioxane as preservative.
2 bottles (62 ml)	3 bottles (62 ml)	32 bottles (62 ml)	**ABTS Substrate Solution** – 2,2'-azino-di-[3-ethylbenzthiazoline-6-sulfonate], containing hydrogen peroxide.
1 bottle (120 ml)	2 bottles (120 ml)	16 bottles (120 ml)	**Stop Solution** – Contains 0.28% sodium fluoride and 0.03% (w/v) bromonitrodioxane as preservative.
10 sheets	40 sheets	400 sheets	**Plate sealers** – Adhesive.
1 each	1 each	4 each	**Clamp and rod** – Closure for foil pack.

Note: The Wash Concentrate and DBS Elution Medium are provided separately and individually as accessories to the kit:

Accessory Product Number	Quantity	Accessory Description
250622	4 bottles (500 ml)	**Wash Concentrate 50X** – Contains 2.5% surfactant in phosphate buffered medium containing 0.05% (w/v) bromonitrodioxane.
250626	4 bottles (120 ml)	**DBS Elution Medium** – Liquid specimen diluent; contains bovine and caprine proteins, salt, surfactant, and anti-microbial agents (contains 0.03% [w/v] bromonitrodioxane).

Note: **Dilsim™ III** and **DBS Elution Medium** contain Dilsim™, a patented diluent.

WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

This test kit is intended for use with serum, plasma, dried blood spots, and oral fluid specimens. Inadequate adherence to package insert instructions may result in erroneous results.

1. Caution: Handle all Avioq HIV-1 Microelisa System biological materials as though capable of transmitting infectious agents. The antigen used to coat the microelisa wells and the positive control sera have been inactivated; nevertheless, both should be handled as though they contain potentially infectious agents. Other components prepared from human serum or plasma have been tested using FDA-licensed tests and found to be nonreactive for the presence of HIV-1 antibody, HTLV-I/II antibody, hepatitis B surface antigen (HBsAg) and HCV antibody. However, as no test method can offer complete assurance that infectious agents are absent, all materials of human origin should be handled as though they contain infectious agents. It has been reported that infectious HIV-1 can be isolated from oral fluid of some infected patients.[16] When detectable in oral fluid specimens, infectious virus is present at low levels compared with blood and may be inactivated by salivary inhibitors.[17]
2. Do not pipet any of the materials by mouth. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
3. Do not perform the test in the presence of reactive vapors (*e.g.*, from sodium hypochlorite, acids, alkalis, or aldehydes) or dust, because the enzymatic activity of the conjugate may be affected.

4. Use disposable gloves. Handle specimens and materials contacting specimens as potentially infectious biological materials in accordance with "Universal Precautions for Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and Bloodborne Pathogens in Health-Care Setting" (CDC, MMWR, June 24, 1988). All test operators should adhere to the Occupational Safety and Health Administration (OSHA) regulations (29 CFR 1910). Consult a physician immediately in the event that contaminated materials are ingested or come in contact with open lacerations, lesions, or other breaks in the skin.
5. Immediately clean up any spillage of material potentially containing antigen or antibody with a 1:10 dilution of 5% sodium hypochlorite. Dispose of the cleaning material by an acceptable method.
6. Dispose of all specimens and materials used to perform the test according to local guidelines. For example:
    a) Autoclave for 60 minutes at 121°C.
    b) Incinerate disposable materials.
    c) Mix liquid waste with 5% sodium hypochlorite solution so that the final concentration is approximately 0.5% sodium hypochlorite. Allow to stand at least 30 minutes before disposal.
7. The **Stop Solution** contains sodium fluoride. Avoid contact with skin. If contact is made, thoroughly wash area with water.
8. OraSure® HIV-1 specimen vials are breakable and should be handled with care.

REAGENT PREPARATION

Prepare all reagents before beginning assay procedure. All reagents and specimens should be at room temperature (15-30°C) before beginning the assay procedure and can remain at room temperature during testing.

Wash Solution

1. Dilute the **Wash Concentrate** 1:50 with purified[14] or NCCLS Type I[15] water in a clean container. Prepare at least 50 ml of **Wash Solution** for each **HIV-1 Microelisa Strip**. Refer to preparation chart.

Preparation of Wash Solution		
Number of Microelisa Strips	**Volume of Wash Concentrate (ml)**	**Volume of Purified Water (ml)**
1-6	16	784
7-12	32	1568

Number of Microelisa Plates	Volume of Wash Concentrate (ml)	Volume of Purified Water (ml)
1	32	1568
2	64	3136
3	96	4704
4	128	6272
5	160	7840
6	192	9408
7	224	10976
8	256	12544
9	288	14112
10	320	15680

The total volume of Wash Solution does not include any additional volume required for an automated washer (priming, dead volume, etc.). Refer to the manufacturer's instructions for the microelisa plate washer.

2. Label the container "**Wash Solution**." Add 7 days to date of preparation and record date on container label, along with the statement “Use before (that date).” Store **Wash Solution** at room temperature.

EnzAbody®

1. Pipet 50 ml **Conjugate Diluent** into 1 vial of **EnzAbody®**. Mix by inverting several times. Avoid excessive foaming. Allow **EnzAbody®** to rehydrate a minimum of 30 minutes before use. Mix again before using to ensure a homogeneous solution. All dye particles should be in solution prior to use.
2. Add 28 days to date of reconstitution and record date on vial label, along with the statement “Use before (that date).” Store prepared **EnzAbody®** at 2-8°C.

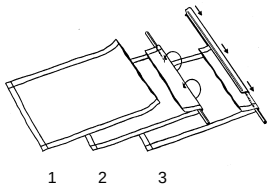
KIT STORAGE INSTRUCTIONS

Store kit reagents at 2-8°C when not in use. The expiration date printed on the kit indicates the date beyond which the product should not be used. Stability of kit reagents after reconstitution or dilution is listed in "REAGENT PREPARATION." Do not store frozen.

HIV-1 Microelisa Strips

The foil packs should be brought to room temperature (15-30°C) before opening to prevent condensation on the **HIV-1 Microelisa Strips**. After the airtight foil pack has been opened the Strips are stable for 4 weeks at 2-8°C if the foil pack is resealed with the clamp and rod or equivalent closure. The silica gel bag must not be removed.

Figure 1: Foil Pack Closure.



Fold open end of foil pack over rod.
Apply clamp.

CHEMICAL OR PHYSICAL INDICATIONS OF INSTABILITY

Alterations in the physical appearance of test kit material may indicate instability or deterioration. The expiration date shown on component labels indicates the date beyond which product should not be used.

SPECIMEN COLLECTION, STORAGE AND SHIPMENT

Collection:

Serum or Plasma

No special preparation or fasting of the patient is necessary. Serum or plasma derived from heparin, citrate, or EDTA (ethylenediaminetetraacetate) as anticoagulants may be used. Serum or plasma separation tubes may be used. Serum or plasma heat inactivated at 56°C for 30 minutes may be used.

Dried Blood Spots

1. Collect newborn blood onto filter paper using the procedure described in the NCCLS publication *Blood Collection on Filter Paper for Neonatal Screening Programs*.[11]
2. Collect adult blood by one of the following methods:
   a) Direct Fingerstick:[11,12] Gently touch the filter paper (Schleicher and Schuell #903 or equivalent) to the drop of blood on the finger.

b) Indirect Fingerstick:[11,12] Collect the blood in a capillary tube and express a drop onto the filter paper. Avoid painting or smearing the blood onto the filter paper with the capillary tube.

c) Venipuncture: Collect a blood sample in an appropriate tube containing anticoagulants (heparin, citrate, or EDTA). Transfer enough blood (approximately 100 to 150 µl) onto the filter paper to fill slightly beyond the printed edge of the circle.[12]

Notes:

With fingersticks, the first drop should be wiped away before collection since the first drop is most likely to contain excess tissue fluid.[11,12]

The blood should soak completely into the filter paper. Prepare one or more spots for each patient. Blood should not be applied to both sides of the filter paper nor should clotted blood be smeared onto the filter paper.

Allow the specimens to dry in a horizontal position without touching any surface for at least three hours.[11] The filter paper may be allowed to dry at room temperature overnight.

3. Do not test dried blood specimens having any of the following characteristics:
    - Foreign substance contamination
    - Blood clots
    - Non-uniform saturation with blood

Oral Fluid Specimens
Refer to the OraSure® HIV-1 Oral Specimen Collection Device package insert for instructions on collecting a specimen.

Storage:

Serum or Plasma
Specimens should be free of microbial contamination and can be stored at 2-8°C for up to 7 days. For long-term storage, specimens should be frozen at –20°C or colder. Specimens repeatedly frozen and thawed more than five (5) times or those containing particulate matter may give erroneous results.

Dried Blood Spots
Dried blood spots may be stored refrigerated (2-8°C), or at room temperature (15-30°C) for 90 days as long as they are not exposed to elevated humidity (>50%). For long-term storage, dried blood spots may be frozen at –20°C or colder at <50% humidity. Although specimens exposed to humidity ≥50% and elevated temperature (37°C) for 14 days did not exhibit a detectable loss in reactivity, Avioq does not recommend routine storage of dried blood spots at elevated temperatures and humidity.

Oral Fluid Specimens
OraSure® HIV-1 specimens (on or off the collection pad) may be stored at 2-37°C for a maximum of 21 days from the time of collection or frozen (-20°C or lower) for 6 weeks. For long term storage, OraSure® HIV-1 specimens may be stored off of the pad in cryovials at –20°C or colder.

Shipment:

Specimens to be shipped must be packaged in compliance with federal regulations governing the transport of etiologic agents.

Dried Blood Spots
For shipment, dried blood spot specimens should be placed in a sealed container, such as a heavy-duty zippered bag with a desiccant. Exposure to greater than 50% relative humidity may adversely affect stability of the specimen.

Oral Fluid Specimens
OraSure® HIV-1 specimens must be transported to the laboratory in the OraSure® HIV-1 specimen vial. Specimens may be transported at ambient temperature.

AVIOQ HIV-1 MICROELISA TEST PROCEDURE

Materials provided

HIV-1 Microelisa Strips
Dilsim™ III
Negative Calibrator Serum (Human)
HIV-1 Positive Control Serum (Human)
Peroxidase Conjugated Goat Anti-human Immunoglobulins (EnzAbody®)
Conjugate Diluent
ABTS Substrate Solution
Stop Solution
Plate sealers
Clamp and rod

Additional materials required but not provided

Instruments/Equipment

Note: For any instrument, the manual provided by the manufacturer should be reviewed for additional information regarding the following:

1. Installation and special requirements.
2. Operation principles, instructions, precautions, and hazards.
3. Equipment calibration.
4. Manufacturer's specifications and performance capabilities.
5. Service and maintenance information.
6. Quality Control.

Automated diluter/dispenser system (minimum 10 µl with 10% accuracy), test tubes, or equivalent

Aspiration/wash system

The aspiration/wash system must be capable of dispensing a minimum volume of 300 µl, and capable of performing a minimum 30 second soak cycle. Aspirated waste must be contained in a closed system.

Adjustable multi-channel variable volume pipet system capable of delivering 50 – 300 µl ± 5%, and tips

Adjustable multi-channel variable volume pipet system capable of delivering 5 – 50 µl ± 5%, and tips

Micropipet(s) capable of delivering, 10µl ± 10%, 500 µl ± 5%, and tips

Incubator

A dry incubator, heating block or equivalent, capable of maintaining 37 ± 2°C.

Microelisa plate reader

Any microelisa reader capable of transmitting light at 405 nm ± 5 nm with a linear absorbance range of 0 to 2.000.

Timer

Graduated cylinder, 50 ml

Reagents/Disposables

Wash Concentrate 50X (Product number 250622)
Clamp and rod (Product number 100004)
Purified water, USP[15] or NCCLS Type I[16] reagent water, or equivalent
Stripholder with uncoated wells (Product number 100003)
Absorbent paper
V-shaped disposable troughs or equivalent
Disposable gloves
Sodium hypochlorite solution (5%) or liquid bleach
Appropriate biohazard waste containers for materials potentially contaminated with infectious agents

Additional materials required but not provided for Dried Blood Spots

DBS Elution Medium (Product number 250626)
Cotton fiber filter paper (Schleicher and Schuell #903 or equivalent)
Lancets
1/4-inch or 1/8-inch hole punch
Plate shaker, rocker or rotator (for room temperature elution)
Zippered plastic bags, Bitran® (Saranex® Series S) plastic bags or equivalent
Uncoated microwell plates for elution (Corning Costar®, Product number 9017 or equivalent)
Desiccant (if necessary)

Procedural notes

1. **HIV-1 Microelisa Strips**, **EnzAbody®**, **Negative Calibrator**, and **Positive Control** used in an assay must be from the same master lot number. Materials should not be used after the expiration date shown on the package label. Components and test specimens should be at room temperature (15-30°C) before testing begins. Return the reagents to 2-8°C after use.

2. **HIV-1 Microelisa Strips** of the microelisa plate are removable. Remove Strips not needed and replace with uncoated Strips. Store unused Strips as described in "KIT STORAGE INSTRUCTIONS." Before testing begins, inspect the microelisa stripholder and ensure that all wells are secure. Stripholders should be handled with care to ensure that no Strip is dislodged during testing. Strips may be numbered to ensure re-insertion should Strips become dislodged.

3. **HIV-1 Microelisa Strips** and plate sealers may be used only once.

4. Do not touch the top or bottom of strips, or the edge of wells with fingers or pipet tips.

5. All reagents and specimens must be mixed well before use. The Positive Control and Negative Calibrator may be vortexed before pipetting. One Positive Control and three Negative Calibrator replicates must be run on each plate (stripholder). If more than one stripholder is processed, ensure that all specified incubation times are met.

6. This assay refers to reagents used to calculate the cutoff for assay results as "**Calibrators**." Instrument printouts and associated literature used in conjunction with this assay may refer to these reagents as "**Controls**." **This difference does not affect assay results.**

7. The **Avioq HIV-1 Microelisa System** utilizes a **Negative Calibrator** to calculate the cutoff value (refer to note below regarding use of an external negative control).

   Note: CLIA regulations require control reagents to be used according to 42 CFR 493. If a bloodborne pathogen test kit uses any of its manufacturer supplied reagents to serve as a **calibrator** function, i.e., either or both of the test kit controls (negative or positive) are used to calculate the assay cutoff, then CLIA regulations require that (an) additional "control" reagent(s) be included in each run. Such reagents may be procured or developed in-house. In any case, prior to placing the additional controls in routine use, each lot of such reagents should have: 1) a known dating period, i.e., validated stability (supplied by a control reagent manufacturer or validated by the user on in-house developed control reagent); and 2) known performance parameters, i.e., specifications for acceptance. Prior to implementation, additional control reagents should be qualified to minimize possible incompatibilities that may exist with particular test kits.

8. Do not allow the microelisa wells to dry once the assay has begun. Fill the wells with the next required reagent immediately after washing; if not possible, fill the wells within 10 minutes. The assay should be repeated if the wells cannot be filled within 10 minutes after washing.

9. Inspect wells after wash steps. Remove any extraneous material on the bottom of any well that could interfere with absorbance reading.

10. All pipetting steps should be performed with the utmost care and accuracy. Use a clean pipet for dispensing specimens and reagents to avoid cross-contamination between reagents, which will invalidate test results. Use micropipets for quantitative delivery of specimens and reagents. For the manual pipetting of controls, calibrator, and specimens, use individual, disposable specimen tips to prevent carryover of specimens. Avoid microbial or any other contamination of reagents.

11. If a specimen is inadvertently not added in this assay, *e.g.*, a well is missed, the assay results for this specimen may be incorrectly interpreted as nonreactive. A missed specimen may be indicated by the lack of color shift in the **Dilsim™ III**.
12. Specimens with microbial contamination may not exhibit a color shift when diluted in **Dilsim™ III**.
13. Minimize opening the door of the incubator during the 37°C incubation time.
14. Avoid chemical contamination of reagents and equipment. Routine maintenance of the aspiration/wash system is strongly recommended to prevent carryover from highly reactive specimens to nonreactive specimens.
15. The aspiration/wash system should be flushed with copious amounts of water upon completion of the final wash of the assay. Use of water containing 0.01% (w/v) bromonitrodioxane will prevent microbial contamination from accumulating when equipment is not in use.
16. Manual plate washing should be validated before use. Use of an automated plate washer is recommended (refer to **Additional materials required but not provided** for automated washer requirements). Incomplete washing may adversely affect the test outcome.
17. Do not return leftover reagents to their original bottles.
18. Do not touch the bottom exterior surface of the microwells. Fingerprints or scratches may interfere with the reading of microwells.
19. Ensure that the Strips are leveled in the stripholder during the test procedure. If necessary, wipe the bottom of the Strips carefully with a soft, lint-free, absorbent tissue to remove any moisture, dust or debris before reading. If necessary, dried buffer may also be removed from the bottom of the Strips with a soft cloth dampened with water, then with a dry, soft, lint-free tissue before reading.
20. **Negative Calibrator** or **Positive Control** values that are not within the expected range (refer to Quality Control section) may indicate a problem with technique, product, or instrumentation.
21. All pipetting equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer's instructions. Consider using dedicated equipment when cross-contamination is a possibility.
22. Bubbles in the Strip wells may cause inaccurate microwell readings. Care should be taken to ensure that no bubbles are present.
23. Use only properly calibrated equipment.
24. When punching disks from dried blood spot specimens, periodically remove accumulated paper fiber residue from the hole punch. This procedure reduces the possibility of cross-contamination from paper dust.
25. With dried blood spot specimens, the paper disk should appear almost white after elution (a faint brown color may remain in the disk).
26. The "Wash procedure" requires a soak cycle of at least 30-seconds.

Wash procedure

1. Incomplete washing will adversely affect the test outcome. Wash Solution must be at room temperature (15-30°C) before use.
2. Aspirate well contents into a waste flask. Then fill the wells completely without overflowing (approximately 0.3 ml) with Wash Solution. Aspirate and fill the wells a total of four times. Allow a minimum of 30-second soak period after each addition of Wash Solution.

   Note: Failure to incorporate these soak periods into the wash procedure may result in increased numbers of falsely reactive specimens.
3. Ensure the Strips are completely aspirated after the final aspiration. If necessary, invert stripholder and tap firmly on absorbent paper to absorb excess Wash Solution. Care should be taken not to dislodge any Strips (gentle pressure applied to the sides of the stripholder during inversion will prevent dislodging of Strips).

Test procedure for serum or plasma specimens
Note: See Dried Blood Spot and OraSure® HIV-1 test procedures for specimen dilutions.

1. Fit stripholder with the required number of **HIV-1 Microelisa Strips**. If less than twelve Strips are needed, use uncoated strips to complete the plate when using a 96-well washer.
2. Prepare a 1:21 dilution of each serum or plasma test specimen, **Calibrator**, and **Control**. Include three wells of Negative **Calibrator**, and one well of **HIV-1 Positive Control** on each run.

   Caution: Use a clean tip for each specimen. Do not pipet specimen into an empty well without **Dilsim™ III**. Do not allow the microelisa wells to dry once the assay has begun.

   a) *Automated diluter/dispenser* : Pipet 10 µl of specimen, **Calibrator**, or **Control** with 200 µl **Dilsim™ III** into the designated microelisa well.

   b) *Premixed manual method* : Pipet 15 µl specimen, **Calibrator**, or **Control** into a clean test tube containing 300 µl **Dilsim™ III**. Mix well. Pipet 210 µl of the diluted specimen into the designated microelisa well.

   c) *Direct manual method* : Using a multichannel pipet, add 100 µl **Dilsim™ III** to each microelisa well. Pipet 10 µl specimen, **Calibrator**, or **Control** into the designated wells. Using a multichannel pipet and clean tips, add an additional 100 µl **Dilsim™ III** to each well and repeatedly aspirate and dispense to mix.

   Note: The addition of a serum or plasma specimen to **Dilsim™ III** will cause the specimen addition indicator to turn the dilution to a lavender color.
3. Cover the Strips with adhesive plate sealers or equivalent. Within 60 minutes of specimen/control addition, incubate Strips at 37 ± 2°C for 60-70 minutes.
4. Wash each well four times with Wash Solution (refer to "Wash procedure") using a soak cycle of at least 30-seconds.
5. Pipet 150 µl of reconstituted **EnzAbody®** working solution into each well.

   Caution: Do not allow **EnzAbody®** to contaminate **ABTS Substrate Solution**. If the same equipment is used to add both reagents, new disposable tips must be used.
6. Cover the Strips with adhesive plate sealers or equivalents. Incubate at 37 ± 2°C for 60 to 65 minutes.
7. Wash each well four times with Wash Solution (refer to "Wash procedure") using a soak cycle of at least 30-seconds.
8. Pipet 150 µl of **ABTS Substrate Solution** into each well. Do not mix or agitate. Do not cover the Strips.
9. Incubate at room temperature (15-30°C) for 10 to 13 minutes.
10. Stop the reaction by adding 150 µl of **Stop Solution** to each well (maintain the same sequence and time intervals used for **ABTS Substrate Solution** addition). Plates should be read within two hours.

11. Blank the microelisa reader on air (without stripholder and Strips) and read the absorbance of the solution in each well at 405 nm.

Test procedure for Dried Blood Spot (DBS) specimens

1. Prepare each dried blood spot specimen using one of the following methods. Ensure that the identity of each specimen is maintained.

    a) *1/4-inch punch* : Punch one disk from each dried blood spot into a designated uncoated strip well. Pipet 150 µl of **DBS Elution Medium** into each well containing a punched dried blood spots and cover the Strips with a new adhesive plate sealer.

    b) *1/8-inch punch* : Punch four disks from each dried blood spot into a designated uncoated strip well. Pipet 150 µl of **DBS Elution Medium** into each well containing punched dried blood spots and cover the Strips with a new adhesive plate sealer.

    Note: To minimize carryover when retesting, the punch may be purged between spots by punching 2-3 clean areas of the filter paper. Remove disks and fibers from the punch by tapping lightly onto an absorbent paper for disposal.

2. Incubate at room temperature (15-30°C) for 60-90 minutes with agitation (400-600 rpm) or overnight (14-22 hours) at 2-8°C.
3. After incubation, fit a stripholder with the required number of **HIV-1 Microelisa Strips**. If less than twelve Strips are needed, use uncoated strips to complete the plate when using a 96-well washer.
4. Pipet 125 µl **Dilsim™ III** into each well of the **HIV-1 Microelisa Strips** that will be used for a dried blood spot specimen.
5. Before addition of DBS eluates to the test plate, mix eluates thoroughly using a plate shaker, or by repeatedly aspirating and dispensing contents with a pipet using a clean tip for each specimen. Transfer 25 µl of each eluate into the **HIV-1 Microelisa Strip** well containing **Dilsim™ III** and mix again.
6. Cover the elution plate containing the remaining dried blood spot eluate with a new plate sealer. Dried blood spot elutions may be stored at 2-8°C for 5 days. For long term storage, eluates may be frozen in microtubes or equivalent at –20°C or colder.
7. Prepare and pipet **Negative Calibrator** and **HIV-1 Positive Control** as described in Step 2 under "Test procedure for serum or plasma specimens."
8. Cover the Strips with adhesive plate sealers or equivalent. Within 60 minutes of specimen/control addition, incubate Strips at 37 ± 2°C for 120-130 minutes.
9. Wash each well four times with Wash Solution (refer to "Wash procedure") using a minimum 30-second soak cycle.
10. Pipet 150 µl of reconstituted **EnzAbody®** working solution into each well.

    Caution: Do not allow **EnzAbody®** to contaminate **ABTS Substrate Solution**. If the same equipment is used to add both reagents, new disposable tips must be used.

11. Cover the Strips with adhesive plate sealers or equivalent. Incubate at 37 ± 2°C for 60 to 65 minutes.
12. Wash each well four times with Wash Solution (refer to "Wash procedure") using a soak cycle of at least 30-seconds.
13. Pipet 150 µl of **ABTS Substrate Solution** into each well. Do not mix or agitate. Do not cover the Strips.
14. Incubate at room temperature (15-30°C) for 10 to 13 minutes.
15. Stop reaction by adding 150 µl of **Stop Solution** to each well (maintain the same sequence and time intervals used for **ABTS Substrate Solution** addition). Plates should be read within two hours.
16. Blank the microelisa reader on air (without stripholder and Strips) and read the absorbance of the solution in each well at 405 nm.

Test procedure for OraSure® HIV-1 specimens

1. Hold the vial upright with the pointed tip up.
2. Move the pad away from the vial tip by gently tapping the vial. Break the pointed tip off the vial.
3. Place a tube over the vial and invert the tube and vial. Centrifuge at 600-800 x g force for 15 minutes.
4. 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.
5. Fit stripholder with the required number of **HIV-1 Microelisa Strips**. If less than twelve Strips are needed, use uncoated strips to complete the plate when using a 96-well washer.
6. Prepare dilutions of each OraSure® HIV-1 specimen.
    a) *Automated diluter/dispenser* : Pipet 75 µl of each OraSure® HIV-1 specimen with 75 µl **Dilsim™ III** into the designated well.
    b) *Direct Manual Method* : Pipet 75 µl of **Dilsim™ III** into the designated microelisa well followed by 75 µl of OraSure® HIV-1 specimen. Mix well by repeatedly aspirating and dispensing contents.
7. Prepare **Negative Calibrator**, and **HIV-1 Positive Control** as described in Step 2 under "Test procedure for serum or plasma specimens."
8. Cover the Strips with adhesive plate sealers or equivalent. Within 60 minutes of specimen/control addition, incubate Strips at 37 ± 2°C for 120-130 minutes.
9. Wash each well four times with Wash Solution (refer to "Wash procedure"), using a minimum 30-second soak cycle.
10. Pipet 150 µl of reconstituted **EnzAbody®** working solution into each well.

    Caution: Do not allow **EnzAbody®** to contaminate **ABTS Substrate Solution**. If the same equipment is used to add both reagents, new disposable tips must be used.
11. Cover the Strips with adhesive plate sealers or equivalent. Incubate at 37 ± 2°C for 60 to 65 minutes.
12. Wash each well four times with Wash Solution (refer to "Wash procedure"), using a minimum 30-second soak cycle.
13. Pipet 150 µl of **ABTS Substrate Solution** into each well. Do not mix or agitate. Do not cover the Strips.
14. Incubate at room temperature (15-30°C) for 10 to 13 minutes.
15. Stop reaction by adding 150 µl of **Stop Solution** to each well (maintain the same sequence and time intervals used for **ABTS Substrate Solution** addition). Plates should be read within 2 hours.
16. Blank the microelisa reader on air (without stripholder and Strips) and read the absorbance of the solution in each well at 405 nm.

QUALITY CONTROL

Qualification of Negative Calibrator (NC) values:

The absorbances of each NC must be greater than or equal to 0.009 and less than or equal to 0.400. Eliminate outliers and calculate the NC mean (NCX). Absorbance of NC must be less than or equal to 1.5 multiplied by NCX and greater than or equal to 0.5 multiplied by NCX. If two or more values are outside range, the run is invalid and must be repeated.

Qualification of HIV-1 (PC) value:

The absorbance value of the PC must be greater than or equal to 0.700. If the PC absorbance value is below this limit, the run is invalid and should be repeated.

TEST VALIDITY

The following criteria must be met in order for the test run to be considered valid:

1. The PC and NCs are qualified.
2. PC - NCX $\geq$ 0.500.

If any of these criteria are not met, technique may be suspect and the run must be repeated.

CALCULATION OF CUTOFF VALUE

Calculate the cutoff value (COV) as follows:

COV = NCX + 0.270

RESULTS

Calculations

Calculations must be made separately for each stripholder. Results are calculated and analyzed the same for serum, plasma, dried blood spots, and OraSure® HIV-1 specimens.

A test specimen is nonreactive if specimen absorbance is less than the cutoff value.

A test specimen is reactive if specimen absorbance is greater than or equal to the cutoff value.

Sample Calculations

Absorbance (example)

NC = 0.175, 0.195, 0.225
NCX = 0.198
PC = 1.469

Acceptance Criteria

Eliminate any calibrator or control absorbance values not meeting the following criteria:
0.009 $\leq$ NC $\leq$ 0.400
NC $\leq$ 1.5 (NCX) or 0.297
NC $\geq$ 0.5 (NCX) or 0.099
PC $\geq$ 0.700

For the above example absorbances, none were eliminated.

Ensure that the difference between the positive control and NCX is acceptable.
PC - NCX $\geq$ 0.500
1.469 - 0.198 = 1.271 Pass
The difference between the positive control and the NCX are within acceptable limits for this example.

Calculate Cutoff Value

COV = NCX + 0.270
COV = 0.198 + 0.270 = 0.468

INTERPRETATION OF RESULTS

Note: Results of the test using an OraSure® HIV-1 specimen must be reported only to the physician who ordered the test or to a person under the supervision of the ordering physician.

In providing test results to the physician, careful note must be taken of the limitations of the procedure (see following section).

1. Specimens with absorbance values less than the cutoff value are considered nonreactive by the **Avioq HIV-1 Microelisa System** criteria and may be considered negative for antibodies to HIV-1. No further testing is required.
2. Specimens with absorbance values greater than or equal to the cutoff value are considered initially reactive by the **Avioq HIV-1 Microelisa System** criteria but before interpretation, the specimen should be retested in duplicate. If either duplicate retest is reactive, the specimen is considered repeatedly reactive.
3. Initially reactive specimens that do not react in both of the duplicate repeat tests are considered negative for antibodies to HIV-1.
4. If the specimen is repeatedly reactive, the probability that antibodies to HIV are present is high, especially in specimens obtained from subjects at increased risk for HIV infection.[13] In addition, persons who have participated in an HIV vaccine study may develop antibodies to the vaccine and may or may not be infected with HIV. In most settings it is appropriate to investigate repeatedly reactive specimens by additional, more specific tests. Specimens found repeatedly reactive by ELISA and positive by additional, more specific tests are considered positive for antibodies to HIV-1. Clinical correlation is indicated with appropriate counseling, medical evaluation and possibly additional testing to decide whether a diagnosis of HIV infection is accurate. If interpretation of results of specimens found repeatedly reactive by ELISA and negative by additional more specific tests is unclear, further clarification may be obtained by testing another specimen obtained three to six months later.

LIMITATIONS OF THE PROCEDURE WITH ORAL FLUID SPECIMENS

1. False negative results occur more frequently when testing OraSure® HIV-1 specimens compared with testing blood specimens. See Performance characteristics section for details.
2. False positive results occur more frequently when testing with OraSure® HIV-1 specimens compared with blood specimens. See Performance characteristics section for details.
3. False negative results (the subject is infected, but the OraSure® HIV-1 Specimen tests negative) may be a result of antibody levels in oral fluid which are below the sensitivity (lower limit of detection) of this procedure which may occur, for example, during the early phase of infection, or with inadequate processing of the specimen, or with inadequate collection of the OraSure® HIV-1 specimen.
4. False positive results may be obtained, for example, as a result of nonspecific cross-reacting antibodies, and not from an HIV-1 infection.
5. False results (either positive or negative) may occur as a result of interfering substances, such as foreign matter in the mouth being collected with the specimen.
6. Reduced sensitivity and specificity of testing with OraSure® HIV-1 specimens compared with testing blood specimens may be observed.

PERFORMANCE CHARACTERISTICS OF THE ASSAY

Reproducibility

Replicates of HIV-1 antibody positive serum, plasma, dried blood spot, or oral fluid specimens with various degrees of reactivity (obtained during a small non-inferiority study), negative specimens, and kit controls were tested at multiple sites (n=3), using multiple kit lots (n=3) and multiple technicians (n=3) on multiple days (n=4). Total, inter-assay, and intra-assay precision is reported in table 1 below. The total coefficient of variation (CV) for serum/plasma and dried blood spot specimens 1-4 ranged from 11.0 to 22.4%. An eight member specimen panel was used for Oral Fluid reproducibility testing. The total coefficient of variation (CV) for oral fluid specimens 1-8 ranged from 19.4 to 25.4%.

Table 1: Assay Reproducibility

				Total		Inter-assay		Intra-assay	
Specimen Type	**ID**	**N**	**Mean**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
Serum or Plasma	1	72	2.78	0.561	20.2	0.533	19.2	0.185	6.7
	2	72	2.67	0.549	20.6	0.472	17.7	0.287	10.8
	3	72	5.48	0.602	11.0	0.538	9.8	0.278	5.1
	4	72	4.77	0.797	16.7	0.711	14.9	0.369	7.7
	NC	108	0.33	0.031	9.4				
	PC	36	4.78	0.732	15.3				
Dried Blood Spots	1	72	1.57	0.272	17.3	0.240	15.3	0.131	8.4
	2	72	1.86	0.416	22.4	0.347	18.7	0.232	12.5
	3	72	3.70	0.782	21.1	0.678	18.3	0.398	10.8
	4	72	3.60	0.676	18.8	0.611	16.9	0.299	8.3
	NC	108	0.34	0.036	10.7				
	PC	36	5.38	0.502	9.3				
Oral Fluid	1	72	3.31	0.644	19.4	0.633	19.1	0.136	4.1
	2	72	0.37	0.079	21.3	0.069	18.7	0.039	10.6
	3	72	1.98	0.503	25.4	0.484	24.5	0.147	7.4
	4	72	0.35	0.071	20.2	0.069	19.5	0.020	5.6
	5	72	2.10	0.479	22.9	0.442	21.1	0.192	9.2
	6	72	1.69	0.399	23.6	0.389	23.1	0.100	5.9
	7	72	1.37	0.284	20.7	0.275	20.1	0.078	5.7
	8	72	1.89	0.437	23.1	0.423	22.4	0.119	6.3
	NC	108	0.33	0.045	13.6				
	PC	36	4.05	0.676	16.7				

ID Specimen Identification
N Number of Replicates
Mean Mean Signal to Cutoff Ratio (SCR)
SD Standard Deviation of SCR
CV Coefficient of Variation of SCR
NC Negative Calibrator
PC HIV-1 Positive Control

Specificity

Specificity was assessed by testing 6,032 serum/plasma specimens and 3,031 dried blood spot specimens collected from three low risk populations, including voluntary blood donors, insurance applicants and Planned Parenthood clinic patients. The licensed Vironostika® HIV-1 Microelisa System was used for comparison. All specimens that were repeatedly reactive with either test were further tested with an FDA licensed Western blot assay.

Of the 6,032 serum/plasma specimens tested, thirteen (13) were repeatedly reactive with this assay and subsequently confirmed HIV-1 antibody positive with a Western blot assay. In contrast, only 12 of the 13 positive specimens were reactive with the comparative test. As summarized in table 2, the assay specificity in this study was estimated to be 100% (6,019/6,019), with a 95% confidence interval of 99.94 - 100%.

Of the 3,031 dried blood spot specimens, thirteen (13) were repeatedly reactive with this assay. These repeatedly reactive specimens matched those of serum specimens, which were confirmed positive with a Western blot assay. The comparative test again detected only 12 of the 13 positive specimens. The specificity for the assay was estimated in this study to be 100% (3,018/3018), with a 95% confidence interval of 99.88 - 100% for dried blood spots.

Table 2: Estimated Specificity for Serum/Plasma and Dried Blood Spot in Low-Risk Populations

		Number tested	Non-reactive	Initially Reactive	Repeatedly Reactive	Western Blot Positive
Serum or Plasma	Population 1	1,500	1,500	0	0	N/A
	Population 2	3,012	3,012	0	0	N/A
	Population 3	1,520	1,506	14	13	13
	Total	**6,032**	**6,018**	**14**	**13**	**13**
Dried Blood Spot	Population 1	0	N/A	N/A	N/A	N/A
	Population 2	1,511	1,511	0	0	N/A
	Population 3	1,520	1,506	14	13	13
	Total	**3,031**	**3,017**	**14**	**13**	**13**

N/A Not applicable

The estimation of specificity is as follows:

$$\frac{\text{(Number screened – Number repeatedly reactive)}}{\text{(Number screened – Number Western blot test positive)}} \times 100$$

Sensitivity

The clinical sensitivity of this assay was evaluated by testing matched serum/plasma specimens and dried blood spot specimens collected from 1,010 HIV-1 infected individuals with various CD4+ counts. These specimens were collected from six sites. Of the 1,010 dried blood spot specimens, 904 (90%) were collected directly as dried blood spots while 106 (10%) were collected with an indirect technique (100 µl of anticoagulated blood spotted onto filter paper).

As summarized in table 3, all serum/plasma specimens and dried blood spot specimens were repeatedly reactive with the assay. Therefore, the sensitivity for both specimen types in this study was 100% (95% CI: 99.64-100%).

Table 3: Estimation of Clinical Sensitivity

Specimen Type	CD4+ Stratum	Number of specimens	Number of Initially Reactive	Number of Repeatedly Reactive
Serum or Plasma	<200	250	250	250
	200-499	385	385	385
	>499	375	375	375
	Total	**1,010**	**1,010**	**1,010**
Dried Blood Spots	<200	250	250	250
	200-499	385	385	385
	>499	375	375	375
	Total	**1,010**	**1,010**	**1,010**

High-risk populations

To assess the performance of the test with specimens collected from high-risk populations, fifteen hundred and fourteen (1,514) specimens were collected from four high-risk populations, which included prison inmates, STD (sexually transmitted diseases) clinic patients, inner city hospital emergency room patients, and HIV-1 outreach clinic patients. In addition, seven hundred and fifty (750) dried blood spot specimens were also collected from two of the study populations.

Serum/plasma specimens were tested with both the licensed Vironostika® HIV-1 Microelisa System for comparison (comparative test) and this assay. If repeatedly reactive with either or both tests, the specimens were further tested with a Western blot assay for confirmation. Dried blood spot specimens were tested only with this assay. Table 4 lists the test results for the assay.

Table 4: High-Risk Populations

Specimen Type	Population	Number of specimens	Number of Initially Reactive	Number of Repeatedly Reactive	Western Blot Positive
Serum or Plasma	1	251	16	14	8
	2	513	13	13	13
	3	500	73	68	68
	4	250	28	27	27
	Total	**1,514**	**130**	**122**	**116***
Dried Blood Spots	1	0	N/A	N/A	N/A
	2	0	N/A	N/A	N/A
	3	500	68	68	68
	4	250	27	27	27
	Total	**750**	**95**	**95**	**95**

*Three repeatedly reactive specimens in population 1 were indeterminate with the Western blot assay due to the presence of only a gp160, a gp120 or a 70 kDa band. The other 3 specimens were not tested by Western blot.

N/A Not applicable

Table 5 shows a summary of the agreement of test results obtained with this assay and the licensed test with specimens from high risk populations. The 6 specimens that were repeatedly reactive (RR) with the assay but non-reactive (NR) with the licensed test were considered to be false positive on the assay. Therefore, the specificity of the assay in this study of high risk populations was calculated to be 1392/1398 = 99.57% (95% CI = 99.07% – 99.84%).

Table 5: Comparison of the Assay with the licensed Vironostika® HIV-1 Microelisa System in testing Specimens from High Risk Populations

		Licensed Test		
		RR	**NR**	**Total**
Assay	**RR**	116	6*	122
	NR	0	1392	1392
	Total	116	1398	1514

*Western blot results were indeterminate for 3 of these specimens. The other 3 specimens were not tested by Western blot.

Seroconversion Panel Testing

Twelve (12) seroconversion panels were tested in triplicate with the assay and the licensed Vironostika® HIV-1 Microelisa System. Due to insufficient amounts of samples, only 10 of the 12 panels were also tested as DBS specimens.

For serum specimens, the assay detects the reactivity earlier than the comparative test in all 12 panels.

Table 6: Seroconversion Panel Testing

		First Reactive Bleed (Days from the First Bleed)			
		Serum		DBS[1]	
Panel ID	**Sample Collection Days**	**Assay**	**Comparative Test[2]**	**Assay**	**Comparative Test[2]**
924	8, 10, 26, 33, 35, 40	33	40	33	NR
927	0, 28, 33, 35, 40	33	40	35	40
931	9, 15, 28, 33, 35, 42	28	33	28	33
932	0, 3, 13, 27, 34, 50, 78, 163, 194	34	NR[3]	78	NR
940	0, 7, 11, 15, 18, 22, 25, 29	15	22	18	25
071	1, 3, 17, 22, 28	17	22	22	28
111	1, 2, 8, 16, 20, 22, 27	8	16	NT[4]	NT
241	1, 7, 9, 15, 17, 22, 24	15	22	17	24
321	1, 8, 12, 15, 21	15	21	15	21
341	1, 7, 10, 21, 23, 28	21	28	28	28
351	1, 8, 11, 15	15	NR	NT	NT
361	1, 3, 9, 11, 16, 18	18	NR	NR	NR

[1]Dried Blood Spot specimens
[2]The licensed Vironostika® HIV-1 Microelisa System was used
[3]None of the specimens in this panel was reactive
[4]Not tested due to limited sample volumes

Dilution Panel Testing

Eight (8) serum specimens representing various HIV-1 group M clades were used to prepare 8 serially diluted panels, which were then tested with the assay and the licensed Vironostika® HIV-1 Microelisa System. Each specimen was tested in duplicate with each of the three kit lots. The first dilutions that gave one or more non-reactive test result in each panel are presented in table 7. Highly diluted serum samples remained reactive with the test for all panels. The assay exhibited higher analytical sensitivity with the dilutional panels for HIV-1 group M clades specimens compared to the comparative test.

Table 7: First dilutions with non-reactive test results for each dilution series

Sample ID	Clade Type	Serum	
		Assay	Comparative Test[1]
5805	HIV-1 Clade B	1:1,920	1:240
H629	HIV-1 Clade B	1:32,000	1:8,000
301-42	HIV-1 Clade A	1:12,800	1:1,600
302-18	HIV-1 Clade C	1:6,000	1:1,500
301-24	HIV-1 Clade D	1:48,000	1:3,000
302-23	HIV-1 Clade E	1:24,000	1:1,500
302-28	HIV-1 Clade F	1:12,800	1:1,600
302-17	HIV-1 Clade D	1:4,000	1:250

[1]The licensed Vironostika® HIV-1 Microelisa System

Detection of HIV-1 Group M Clades

Seventy-two (72) specimens representing infections with various clades of HIV-1 group M viruses were tested in duplicate with the assay. Since there were limited amounts for most specimens, all specimens were diluted by 100 to 10,000 times prior to testing. Table 8 shows the test results for serum/plasma specimens. All specimens were repeatedly reactive with the assay, despite the dilution factors.

Table 8: Detection of HIV-1 Group M Clades

Clade	Number of Specimens	Sample Dilution Factor	Number of Repeatedly Reactive	Number of Non-reactive
A	10	100 to 10,000	10	0
B	12	100 to 10,000	12	0
B/D	1	100	1	0
C	10	100 to 10,000	10	0
C/E	1	1,000	1	0
D	10	100 to 10,000	10	0
E	7	100 to 5,000	7	0
E/A	3	1,000	3	0
E/C	1	10,000	1	0
E/F	1	100	1	0
F	10	100 to 10,000	10	0
G	4	1,000 to 5,000	4	0
H	2	1,000	2	0
Total	**72**		**72**	**0**

Detection of HIV-2 Positive Specimens

Twenty (20) HIV-2 positive serum/plasma specimens were diluted and tested in duplicate with the assay. All HIV-2 specimens were repeatedly reactive with the assay. The test results are listed below in table 9.

Table 9: Detection of HIV-2 Specimens

Specimen ID	Sample Dilution	Mean SCR*
1	1:10	3.3
2	1:10	5.8
3	1:500	1.5
4	1:10	4.3
5	1:50	1.7
6	1:10	5.5
7	1:10	2.5
8	1:10	5.0
9	1:100	3.7
10	1:1,000	1.8
11	1:200	1.3
12	1:200	1.4
13	1:10	1.9
14	1:50	1.3
15	1:1,000	4.9
16	1:1,000	3.6
17	1:50	1.7
18	1:1,000	2.7
19	1:10	2.0
20	1:10	1.8

*Specimens with SCR equal to or greater than 1.0 are considered reactive with the test.

Reactivity with Potentially Interfering Substances or Medical Conditions
Serum or plasma specimens (S/P)

Samples were collected from individuals with medical conditions that may cause nonspecific assay reactivity. These specimens were tested using the assay. As shown in table 10, all but one specimen (184/185) were non reactive with the assay. The one specimen weakly reactive with the assay was from an individual with elevated bilirubin. Further analysis of this specimen with a Western blot assay showed the presence of antibodies weakly reactive with gp160. Therefore, elevated bilirubin was unlikely the cause for the reactivity of the specimen. Rather, the presence of nonspecific antibody in the sample contributed to the weak reactivity.

In a second study, the 184 non-reactive samples were spiked with a small volume of HIV-1 positive sample and tested with the assay. All specimens were reactive with the assay. The medical conditions listed in table 10 did not affect the reactivity of specimens spiked with HIV-1 seropositive sample.

Dried blood spot specimens (DBS)

A similar study was also performed using dried blood spot specimens. As shown in table 10, all 184 specimens were non-reactive with the assay. When spiked with a small volume of HIV-1 seropositive sample, all 184 specimens were reactive with the assay. No interference was observed with dried blood spot specimens.

Table 10: Reactivity of Specimens from Individuals with Potentially Interfering Substances or with Medical Conditions

Specimen type	Number of specimens		Neat Specimens				Spiked Specimens			
			No. of Reactive		No. of non-reactive		No. of Reactive		No. of non-reactive	
	S/P	DBS	S/P	DBS	S/P	DBS	S/P	DBS	S/P	DBS
Antinuclear antibody positive	10	10	0	0	10	10	10	10	0	0
CMV antibody positive	10	10	0	0	10	10	10	10	0	0
EBV antibody positive	10	10	0	0	10	10	10	10	0	0
Rubella antibody positive	9	9	0	0	9	9	9	9	0	0
Elevated bilirubin[1]	10	10	1	0	9	10	9	9	0	0
Hemolysed specimens	10	10	0	0	10	10	10	10	0	0
HSV-1 or HSV-2 antibody positive	10	10	0	0	10	10	10	10	0	0
HTLV-I or HTLV-II antibody positive	10	10	0	0	10	10	10	10	0	0
Lipemic[2]	10	10	0	0	10	10	10	10	0	0
Multiple transfusion	10	10	0	0	10	10	10	10	0	0
Multiparous females	10	10	0	0	10	10	10	10	0	0
Rhematoid factor positive	10	10	0	0	10	10	10	10	0	0
SLE positive	10	10	0	0	10	10	10	10	0	0
Syphilis antibody positive	10	10	0	0	10	10	10	10	0	0
Toxaplasmosis gondii positive	7	7	0	0	7	7	7	7	0	0
HBV antigen positive	10	10	0	0	10	10	10	10	0	0
HCV antibody positive	10	10	0	0	10	10	10	10	0	0
Hypergammaglobulinemia	9	8	0	0	9	8	9	8	0	0
Influenza vaccinated	10	10	0	0	10	10	10	10	0	0
Total	**185**	**184**	**1**	**0**	**184**	**184**	**184**	**184**	**0**	**0**

[1] >6.3 mg/dL
[2] >980 mg/dL

PERFORMANCE CHARACTERISTICS OF THE ASSAY FOR THE ORAL FLUID SPECIMENS COLLECTED WITH THE ORASURE® CELLECTION DEVICE

Non-Inferiority Study

A small non–inferiority study was performed between Avioq HIV-1 Microelisa System and the licensed comparator Oral Fluid Vironostika® HIV-1 Microelisa System. The study indicated that the Avioq HIV-1 Microelisa System was not inferior to that of the licensed comparator Oral Fluid Vironostika® HIV-1 Microelisa System.

ORIGINAL ORAL FLUID PERFORMANCE CHARACTERISTICS

All Performance Characteristics presented below are from the licensed Oral Fluid Vironostika® HIV-1 Microelisa System.

Sensitivity and specificity: Sensitivity testing of OraSure® HIV-1 specimens with the Oral Fluid Vironostika® HIV-1 Microelisa System was computed based on the clinical diagnosis of AIDS and specificity was computed based on testing in low risk populations. In addition, sensitivity and specificity of OraSure® HIV-1 testing were computed based on testing in high risk subjects using matched oral fluid/blood specimens from the same subjects.

1. Sensitivity using OraSure® HIV-1 specimens, based on an assumed 100% prevalence of HIV-1 antibody in AIDS patients, is estimated in these studies to be 98.6% (287/291). Sensitivity using OraSure® HIV-1 specimens was reduced compared with blood specimens in AIDS patients.

   Sensitivity using OraSure® HIV-1 specimens is estimated in these studies to be 99.1% (546/551) in high risk subjects, based on the ability of the test to detect HIV-1 antibody in matched oral fluid/blood specimens. Sensitivity using OraSure® HIV-1 specimens was reduced compared with blood specimens for high risk subjects.

2. Specificity using OraSure® HIV-1 specimens, based on an assumed zero prevalence of HIV-1 antibody in low risk populations, is estimated in these studies to be 99.6% (3991/4009). Specificity using OraSure® HIV-1 specimens was reduced compared with blood specimens for low risk subjects.

   Specificity using OraSure® HIV-1 specimens is estimated in these studies to be 97.7% (837/857) for high risk subjects, based on the ability of the test to detect HIV-1 antibody in matched oral fluid/blood specimens. Specificity using OraSure® HIV-1 specimens was reduced compared with blood specimens for high risk subjects.

Clinical studies

Clinical studies of matched OraSure® HIV-1 and serum specimens from AIDS patients, high risk subjects and low risk subjects 18 years of age and older were conducted at seven sites as shown in the following table.

Test Site	AIDS Subjects	High Risk Subjects	Low Risk Subjects
A	11	407	84
B	0	466	0
C	158	299	1,132
D	65	240	104
E	57	0	336
F	0	0	573
G	0	0	1,788
Total	291	1,412	4,017

Clinical sensitivity studies

Reactivity in AIDS patients and high risk populations

The sensitivity of testing OraSure® HIV-1 specimens compared with matched serum specimens using the Oral Fluid Vironostika® HIV-1 Microelisa System in AIDS patients and high risk subjects (38% intravenous drug users, 23% homosexuals, 17% sexual partners of individuals at risk, 6% prostitutes, 16% others with acknowledged risk factors) is shown in the table that follows.

	No. of Specimens	Nonreactive[a] No.	Reactive No.	Number Confirmed Positive with Serum
AIDS Patients				
OraSure® HIV-1	291	4[b]	287	
Serum	291	1	290	291
High Risk Subjects				
OraSure® HIV-1	1,412	843[c]	569[d]	
Serum	1,412	858	554[e]	551

[a]Includes specimens that were nonreactive on the initial screening test and specimens that were initially reactive, but not repeatedly reactive.

[b]Four matched serum specimens were positive (OraSure® HIV-1 False Negative). Screening tests for 2 patients were valid by ELISA kit criteria and the matched serum specimens were positive with S/CO of 7.26 and 6.90, but laboratory control reagents failed. Retests of these OraSure® HIV-1 specimens at the clinical site were positive for both specimens with S/CO values of 1.69 and 1.15.

[c]Five matched serum specimens were positive (OraSure® HIV-1 False Negative).

[d]Twenty matched serum specimens were negative (OraSure® HIV-1 False Positive); 3 OraSure® HIV-1 specimens were IR and not retested and matched serum specimens were negative (OraSure® HIV-1 Unresolved).

[e]Two subjects were WB^{neg} (Blood False Positive); 1 subject was IR, not retested and WB^{ind} (Unresolved).

ELISA = Oral Fluid Vironostika® HIV-1 Microelisa System assay; IR = initially reactive; RR = repeatedly reactive; WB = blood Western blot; pos = positive; ind = indeterminate; neg = negative; RIPA = radioimmunoprecipitation assay; S/CO = signal to cutoff

Out of the 291 AIDS patient studies, 287 OraSure® HIV-1 specimens (98.6%) and 290-matched serum specimens (99.7%) were reactive in the Oral Fluid Vironostika® HIV-1 Microelisa System screening. Of the four OraSure® HIV-1 specimens that were initially nonreactive, one was nonreactive when retested and three were reactive when retested. One serum specimen that was initially nonreactive was reactive when retested.

Out of the 551 high risk subjects whose serum tested positive in additional, more specific tests for HIV-1 antibodies (Western blot, RIPA), 546 OraSure® HIV-1 specimens (99.1%) and 551 matched serum specimens (100%) were reactive in the Oral Fluid Vironostika® HIV-1 Microelisa System screening test. Of the five OraSure® HIV-1 specimens that were initially nonreactive, two were repeatedly nonreactive on ELISA retest, two were not retested and one was repeatedly reactive on retest.

The sensitivity of testing OraSure® HIV-1 specimens with the Oral Fluid Vironostika® HIV-1 Microelisa System compared with matched serum specimens was reduced based on the reactivity in AIDS patients and high risk subjects.

Analytical sensitivity

The analytical sensitivity of testing OraSure® HIV-1 specimens with the Oral Fluid Vironostika® HIV-1 Microelisa System was 1/1000th that of testing serum specimens based on serial dilution of matched specimens from 13 HIV-1 antibody positive subjects. At the dilutions recommended for testing, 1:2 for OraSure® HIV-1 specimens and 1:75 for serum specimens, the average sensitivity of testing with OraSure® HIV-1 specimens was 1/35th (range 1/7 to 1/107) that of testing with serum specimens based on the highest dilution producing positive results as shown in the following table.

Specimen Number[a]	OraSure® HIV-1 Titer[c]	Serum Titer[c]	OraSure® HIV-1 Hemoglobin[b] (mg/dl)
1	128	4,096	
2	128	4,096	
3	64	4,096	
4	64	1,024	
5	128	4,096	
6	128	4,096	
7	128	4,096	
8	512	4,096	
9	128	4,096	
10	512	4,096	
11	160	17,067	11.0
12	1,280	8,533	7.6
13	160	8,533	5.3

[a]Matched specimens 1-10 were collected from analytical study. Matched specimens 11-13 were collected as part of the clinical field trial.

[b]Three OraSure® HIV-1 specimens (#11-13) of 112 studies contained hemoglobin (≥ 5 mg/dl).

[c]End-point titer indicates the maximum dilution of a specimen (prior to dilution for ELISA testing) which produced positive Oral Fluid Vironostika® HIV-1 Microelisa System test results.

The analytical sensitivity of Oral Fluid Vironostika® HIV-1 Microelisa System for HIV-1 antibodies in three OraSure® HIV-1 specimens studied was not enhanced by the presence of blood in OraSure® HIV-1 specimens.

Reactivity in seroconversion

OraSure® HIV-1 and serum specimens were obtained prospectively from one subject undergoing seroconversion. Results of ELISA testing show that in this case antibodies to HIV-1 were detected in serum specimens on day 8 and OraSure® HIV-1 specimens on day 11 based on two consecutive positive determinations. Reactivity of ELISA using serum and OraSure® HIV-1 specimens and WB and HIV-1 antigen tests using serum is summarized in the following table.

Date	(Day)	OraSure® HIV-1 S/CO	Blood S/CO	Blood p24 Ag (ng/ml)	Blood Western blot band reactivity
5/14	(1)	0.36	0.40	13.64*	none
5/15	(2)	0.57			
5/16	(3)	0.31	0.40	20.36*	none
5/17	(4)	0.52			
5/21	(8)	0.49	2.92*	6.43*	$gp160^{+/-}$ $p24^{+/-}$*
5/22	(9)	1.07*			
5/23	(10)	0.96	4.71*	1.92*	$gp160^{+/-}$ $p24^{+}$ $gp41^{+/-}$*
5/24	(11)	1.75*			
5/28	(15)	1.94*	5.26*	0	$gp160^{+}$ $p24^{+}$ $gp41^{+/-}$ $gp120^{+/-}$*
5/29	(16)	2.05*			
5/30	(17)	2.04*	5.27*	0	$gp160^{+}$ $p24^{+}$ $gp41^{+/-}$ $gp120^{+/-*}$
5/31	(18)	2.04*			
6/4	(22)	1.91*	5.44*	0	$gp160^{+}$ $p24^{+}$ $gp41^{+/-}$ $gp120^{+/-}$ $p66^{+/-}$ $p55^{+/-}$*

*Asterisk indicates a reactive test result; S/CO = signal to cutoff; Ag = HIV-1 antigen; + = positive reactivity; +/- = indeterminate reactivity

Reactivity in seropositive subjects with oral pathology

Oral examinations were carried out at clinical site D on 65 AIDS subjects, 240 high risk subjects and 18 subjects of unknown risk. Of the 303 subjects found to be seropositive, 29 (10%) had significant oral pathology. For these subjects HIV-1 antibodies were detected in 96.6% (28/29) of the OraSure® HIV-1 specimens and 100% (29/29) of the serum specimens when tested using the Oral Fluid Vironostika® HIV-1 Microelisa System as shown in the following table.

Oral Pathology	Number Tested	Number OraSure® HIV-1 ELISA Positive	Number Confirmed Positive with Serum
Hairy Leukoplakia	10	10	10
Candida	10	9[a]	10
Gingivitis	5	5	5
Gingival Ulcer	1	1	1
Periodontitis	1	1	1
Hairy leukoplakia and candida	1	1	1
Hairy leukoplakia and gingivitis	1	1	1
Total	29	28	29

[a]Includes one OraSure® HIV-1 false negative which was previously noted in high risk populations.

This study does not detect an increase in the frequency of false negative results for OraSure® HIV-1 specimens obtained from subjects with the above oral pathologies.

Clinical specificity studies

Specificity in low risk and high risk populations

The specificity of testing OraSure® HIV-1 specimens with the Oral Fluid Vironostika® HIV-1 Microelisa System compared to testing serum specimens was studied in 4017 low risk subjects (55% military inductees, 16% blood donors, 14% insurance applicants, 11% students/hospital staff) and 1412 high risk subjects. The results are shown in the table that follows:

	No. of Specimens	Nonreactive[a] No.	Reactive No.	Number Confirmed Positive with Serum
Low Risk Subjects				
OraSure® HIV-1	4,017	3,992	25[b]	
Serum	4,017	4,008	9[c]	4
High Risk Subjects				
OraSure® HIV-1	1,412	843	569[d]	
Serum	1,412	858	554[e]	551

[a]Includes specimens that were nonreactive on the initial screening test and specimens that were initially reactive, but not repeatedly reactive.

[b]Eighteen matched serum specimens were negative (OraSure® HIV-1 False Positive); 3 OraSure® HIV-1 specimens were IR and not retested and matched serum specimens were ELISA^{neg} and WB^{ind} (Unresolved).

[c]Four specimens were confirmed negative (Blood False Positive); 1 specimen was RR, but not tested further (Unresolved).

[d]Twenty matched serum specimens were negative (OraSure® HIV-1 False Positive); 3 matched serum specimens were negative and OraSure® HIV-1 was IR and not retested. 19/20 subjects with false positive OraSure® HIV-1 results were smokers.

[e]Two specimens were WB^{neg} (Blood False Positive); 1 specimen was ELISA IR and WB^{ind} (Unresolved).

ELISA = Oral Fluid Vironostika® HIV-1 Microelisa System assay; IR = initially reactive; RR = repeatedly reactive; WB = blood Western blot; pos = positive; ind = indeterminate; neg = negative; RIPA = radioimmunoprecipitation assay; S/CO = signal to cutoff

There were 18 repeatedly reactive OraSure® HIV-1 specimens (18/4009, 0.45%) compared with 4 repeatedly reactive serum specimens (4/4009, 0.10%) in the low risk subjects whose HIV antibody status was resolved to be negative by additional testing. There were 20 repeatedly reactive OraSure® HIV-1 specimens (20/857, 2.3%) compared with 2 repeatedly reactive serum specimens (2/860, 0.23%) in the high risk subjects whose antibody status was resolved to be negative by additional testing.

These results suggest that, compared with testing serum specimens, the incidence of false positive test results using OraSure® HIV-1 specimens is increased 4.5-fold in low risk populations and 10-fold in high risk populations.

Reactivity in seronegative subjects with oral pathology

Matched OraSure® HIV-1 and serum specimens were obtained prospectively from 47 subjects with various forms of oral pathology. Results of testing OraSure® HIV-1 and serum specimens with Oral Fluid Vironostika® HIV-1 Microelisa System were negative in all cases as shown in the following table.

Oral Pathology	Number Tested	Number Positive	Number Negative
Periodontitis	21	0	21
Gingivitis	5	0	5
Multiple caries	5	0	5
Multiple caries and periodontitis	8	0	8
Multiple caries and gingivitis	6	0	6
Periodontitis and gingivitis	2	0	2
Total	47	0	47

This study did not detect differences in specificity among the OraSure® HIV-1 specimens obtained from subjects with the oral pathology noted above when tested in the Oral Fluid Vironostika® HIV-1 Microelisa System.

Reactivity in other disease conditions

Thirty-eight repository and 53 fresh matched OraSure® HIV-1 and serum specimens from patients with medical conditions other than HIV-1 infection were studied. ELISA testing of OraSure® HIV-1 and serum specimens were negative in all cases as shown in the following table.

Disease	Number Tested	Number Positive	Number Negative
Hepatitis A	12	0	12
Hepatitis B	5	0	5
Hepatitis C	18	0	18
Autoimmune	13	0	13
H. pylori	3	0	3
Lymphoid malignancy	16	0	16
Other neoplasia	24	0	24
Total	91	0	91

Additional performance studies

The testing of OraSure® HIV-1 specimens collected by trained medical professionals and trained non-medical individuals compared with testing of the corresponding serum specimens were evaluated using the Oral Fluid Vironostika® HIV-1 Microelisa System. Matched OraSure® HIV-1 and serum specimens were obtained from 129 subjects prospectively and the results of testing are presented in the table that follows.

Trained Collector	Specimen	Number Negative	Number Positive
Medical professional	OraSure® HIV-1	94	35[a]
Non-medical	OraSure® HIV-1	95	34[b]
Medical professional	Serum	96	33[c]

[a]Three matched sera were negative (OraSure® HIV-1 False Positive).

[b]Two matched sera were negative (OraSure® HIV-1 False Positive) and coincided with those identified in footnote a above.

[c]One serum was ELISA^{RR} and WB^{ind} with p65 reactivity (Unresolved).

Positive ELISA results for OraSure® HIV-1 specimens collected by trained medical professionals and non-medical individuals from 32 subjects were concordant with serum results. Discordant results were obtained for one subject whose OraSure® HIV-1 specimen was ELISA repeatedly negative and serum specimen was ELISA repeatedly reactive and Western blot indeterminate with p65 reactivity. There was no follow up testing to resolve the true serostatus of this subject.

Three OraSure® HIV-1 specimens collected by medical professionals and two OraSure® HIV-1 specimens collected by non-medical individuals were repeatedly reactive on the ELISA test while matched serum specimens were negative on ELISA and WB tests (OraSure® HIV-1 false positive).

In this study, the performance of ELISA testing of OraSure® HIV-1 specimens collected by trained non-medical individuals was comparable to OraSure® HIV-1 specimens collected by medical professionals.

Reproducibility

The reproducibility of testing, OraSure® HIV-1 specimens in the Oral Fluid Vironostika® HIV-1 Microelisa System was studied and is summarized in the following table.

Specimen	Mean S/CO	% CV	Test n	Test Days	Location A	Location B	Epitope
1	6.046	2.3	36	2	X		
2	6.018	2.4	36	2	X		
3	5.933	5.9	33	2	X		
4	6.142	2.6	5	2	X		
5	2.852	10.6	24	3		X	X
6	1.639	29.6	10	3	X	X	
7	0.422	23.7	82	3	X	X	
8	0.299	14.4	88	3	X	X	

OraSure® HIV-1 specimens from four seropositive subjects (specimens 1-4) and two seronegative subjects (specimens 7 and 8) were collected at two test sites. Specimens collected at one clinical site were tested on-site and exchanged with the other site for testing, where indicated. OraSure® HIV-1 specimens from two seropositive subjects were collected and diluted at Epitope to provide one intermediate (specimen 5) and one low positive specimen (specimen 6) for testing. Specimens prepared at Epitope were sent to clinical sites for testing and/or tested at Epitope as indicated.

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AVAILABILITY

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