VioOne™ HIV Profile™ Supplemental Assay

Key Symbols Used

	Catalogue Number		Consult Instructions For Use
	Batch Code		In Vitro Diagnostic Medical Device
	Expiration Date		Positive Control
	Temperature Limit		Negative Control
	Latex Free		Contains Sufficient For <n> Tests
	Caution		Keep Away from Sunlight
			Biological Risk

VioOne™ HIV Profile™ Supplemental Assay

Serologic assay for detection and differentiation of antibodies directed to different gene products of HIV-1 and HIV-2

Store between 2-8°C.

For In Vitro Diagnostic Use

INTENDED USE

The VioOne™ HIV Profile™ Supplemental Assay is an enzyme-linked immunosorbent assay (ELISA) for confirmation and differentiation of individual antibodies directed to various gene products of Human Immunodeficiency Virus Type 1 (HIV-1 Group M & Group O) and Type 2 (HIV-2) in human serum or plasma. The HIV Profile™ is intended as an aid in the diagnosis of infection with HIV-1 and/or HIV-2. It is intended as an additional, more specific test to confirm the presence of antibodies to HIV-1 and HIV-2 for specimens repeatedly reactive in diagnosis or screening procedures, including pediatric patients (ages 2-20). Results of the HIV Profile™ can also be used to distinguish recent from longstanding HIV-1 infection and thus used for HIV-1 incidence estimation.

SUMMARY AND EXPLANATION OF THE TEST

Published data indicate a strong correlation between the acquired immunodeficiency syndrome (AIDS) and infection with the retrovirus 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, antibodies to viral antigens appear in blood specimens from infected individuals, a process known as seroconversion. After seroconversion, HIV specific antibodies can be readily detected in blood specimens. Current procedure for diagnosis of an HIV infection requires that a repeatedly reactive sample with a diagnostic or screening assay be confirmed using a more specific assay, which is commonly known as a confirmatory test. The HIV Profile™ Supplemental Assay was developed as a confirmatory test to detect and distinguish between antibodies to HIV-1 *env*, *pol*, and *gag* gene products as well as antibodies to HIV-2.

Recent recognition that information on HIV incidence, *i.e.*, the proportion of new HIV infections in a population, is important for public health purposes in that HIV incidence can be used to monitor HIV epidemics, improve the intervention approaches for the targeted population, and evaluate the effectiveness of HIV prevention and treatment programs. Because all repeatedly reactive samples for HIV antibodies must be confirmed using a confirmatory assay, incorporation of HIV incidence detection into a confirmation assay provides an effective and economic means for determination of HIV incidence.

The VioOne™ HIV Profile™ Supplemental Assay was incorporated with a means to differentiate a recent infection from a longstanding infection, which in turn may be used to estimate HIV incidence in a population. Signal to cutoff result from two solid phase wells, one coated with normal concentration of HIV-1 gp160 and the other coated with a reduced concentration of HIV-1 gp160, is used to calculate a Recency Index (RI) that is used to differentiate a recent infection from a longstanding infection, which in turn may be used to estimate HIV-1 incidence in a population.

PRINCIPLE OF THE TEST

This test uses HIV-1 *env*, *pol*, and *gag* gene products as recombinant antigens and an HIV-2 specific transmembrane peptide (gp36). These antigens are individually coated onto the wells of microwell plate strips (solid phase). Upon addition of a diluted test specimen, antibodies to HIV-1 or HIV-2, if present, form immune complexes through the interaction between anti-HIV-1 or anti-HIV-2 antibodies in the specimen and HIV-1/HIV-2 antigens coated on microwells. The Sample Diluent contains biotinylated HIV-1 p24 antigen. If present, HIV-1 p24 antibody captured by solid phase HIV-1 p24 antigen also binds the biotinylated HIV-1 p24 antigen. Following

incubation, the specimen / Sample Diluent mix containing biotinylated HIV-1 p24 antigen is aspirated and microwells are washed with buffer. Subsequently, Conjugate containing horseradish peroxidase (HRP)-labeled neutravidin and HRP-labeled HIV-1 and HIV-2 antigens is added to all microwells. Peroxidase-labeled neutravidin binds to any biotinylated *gag* antigen / antibody complexes while HRP-labeled HIV-1 and HIV-2 antigens bind to any HIV-1 or HIV-2 antibodies captured on the solid phase. Following an aspiration and wash to remove excess Conjugate and incubation with TMB (Tetramethylbenzidine) substrate, a blue color is produced. The enzyme reaction is stopped by the addition of a sulfuric acid solution, which changes the color to yellow. The amounts of HIV specific antibodies in specimens, if any, are proportional to color intensity.

Components in each VioOne™ HIV Profile™ Kit

24 Tests	Component Description
2 stripholders	**Microelisa Strips** – Twelve per holder, contained in a re-sealable foil pouch with silica gel desiccant. Each strip contains 8 wells coated with no viral antigen, HIV-1 antigens, and HIV-2 antigens contained in a foil pouch with silica gel desiccant.
1 bottle (25 ml)	**Sample Diluent** – Liquid specimen diluent with biotinylated HIV-1 p24 antigen; contains animal proteins, salt, surfactants, Patent Blue V as coloring reagent, and (0.03% (w/v) bromonitrodioxane as preservative.
1 vial (1.0 ml) CONTROL −	**Negative Control Serum (Human)** – Contains human serum with protein stabilizers and 0.05% (w/v) bromonitrodioxane as preservative; nonreactive to HBsAg and HIV-1 antigen, antibodies to HIV, HTLV-I/II, and HCV.
1 vial (1.0 ml) CONTROL +	**HIV-1/2 Positive Control Serum (Human)** – Inactivated human serum containing protein stabilizers. Contains 0.05% (w/v) bromonitrodioxane as preservative and Amaranth as coloring agent; reactive for antibodies to HIV-1 / HIV-2.
2 vials	**Conjugate** – Lyophilized, horseradish peroxidase conjugated NeutrAvidin, HIV-1 antigens, and HIV-2 antigens with protein stabilizers and Amaranth.
1 bottle (55 ml)	**Conjugate Diluent** – Phosphate buffered saline containing protein stabilizers and 0.03% (w/v) bromonitrodioxane as preservative.
1 bottle (22 ml)	**TMB Solution** – Citric acid containing 0.03% (w/v) tetramethylbenzidine.2HCl.
1 bottle (22 ml)	**Peroxide Solution** – Citric acid/sodium citrate buffer containing 0.04% urea peroxide.
10 sheets	**Plate sealers** – Adhesive.

Note:

1. Wash Buffer Concentrate is provided as an accessory to the kit (500 mL/bottle). Do not use any other Wash Buffer for this assay. Wash Buffer Concentrate is stored at room temperature.
2. The Stop Solution is 2N Sulfuric Acid and is not provided by Avioq, Inc. Do not use any other Stop Solution for this assay.
3. NeutrAvidin is a trademark of ThermoFisher

WARNINGS AND PRECAUTIONS

This test kit is not intended for screening of Blood donors.

This test kit is intended for use with serum or plasma. Inadequate adherence to package insert instructions may result in erroneous results.

1. Caution: Handle all VioOne™ HIV Profile™ Supplemental Assay biological materials as though capable of transmitting infectious agents. Positive control sera have been inactivated but 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 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.
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.

    Note: Liquid waste containing acid must be neutralized prior to the addition of disinfectants and/or disposal.
7. Some components of this kit contain small concentrations of hazardous chemicals (TMB Solution and Peroxide Solution).
8. 2N Sulfuric Acid used as stop solution is corrosive and should be handled with care to prevent exposure to skin and eyes. If this reagent comes into contact with skin or eyes, wash thoroughly with water.

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. Check the **Wash Buffer Concentrate** for the presence of crystals or precipitate. If crystals or precipitate have formed in the solution, resolubilize by warming at 37ºC until crystals or precipitate dissolve. **Wash Buffer Concentrate** may appear slightly cloudy or show some phase separation after warming which is acceptable. Mix the **Wash Buffer Concentrate** before diluting.

2. Dilute the **Wash Buffer Concentrate** 1:7.5 with purified[6] water in a clean container. Prepare **Wash Solution** for each strip or plate according to the following preparation chart:

Preparation of Wash Solution		
Number of Microelisa Strips	**Volume of Wash Concentrate (mL)**	**Volume of Purified Water (mL)**
1-6	120	780
7-12	240	1560

Number of Microelisa Plates	**Volume of Wash Concentrate (mL)**	**Volume of Purified Water (mL)**
1	240	1560
2	480	3120

The total volume of Wash Solution includes additional volume for an automated washer (priming, dead volume, etc.). Refer to the manufacturer's instructions for the plate washer.

3. 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.

Preparation of Conjugate Concentrate

1. Pipet 1 mL **Conjugate Diluent** into one vial of **Conjugate**. Mix the contents thoroughly but avoid excessive foaming. Allow **Conjugate Concentrate** to rehydrate a minimum of 30 minutes prior to use. Do not handle **Conjugate Concentrate** with gloves that have come into contact with serum or plasma.
2. Record reconstitution date on each vial. Proceed to preparation of Working Conjugate Solution or store reconstituted **Conjugate Concentrate** at 2-8°C. The stability of reconstituted **Conjugate Concentrate** is 14 days of stability at 2-8°C.

Preparation of Conjugate Working Solution

1. Clean, preferably disposable/dedicated, polypropylene vessels should be used. **Do not use polystyrene containers**. Ensure reconstituted **Conjugate Concentrate** is well mixed and at room temperature before use. Transfer an appropriate amount of **Conjugate Diluent** to a vessel and add an appropriate amount of reconstituted **Conjugate Concentrate** to make a 1:12 **Conjugate Working Solution** (see table below). Return unused reconstituted **Conjugate Concentrate** to 2-8°C.

Preparation of Conjugate Working Solution

Number of Microelisa Strips	**Volume of Reconstituted Conjugate Concentrate**	**Volume of Conjugate Diluent**
3	0.25 mL	2.75 mL
6	0.50 ml	5.50 mL
9	0.75 mL	8.25 mL
12	1.00 mL	11.00 mL

2. Once prepared, **Conjugate Working Solution** is stable for four hours at room temperature. Discard any unused **Conjugate Working Solution** after four (4) hours.

Preparation of TMB Substrate 

Prepare **TMB Substrate** in a clean, preferably disposable/dedicated, polypropylene container. **Do not use polystyrene containers**. Transfer a sufficient amount of **Peroxide Solution** to a container, add an equal amount of **TMB Solution** to the **Peroxide Solution** and mix thoroughly prior to use (see table below).

Each microwell plate requires at least 10 ml of **TMB Substrate**. More **TMB Substrate** may be needed depending upon the reagent dispenser used. See the instrument manufacturer's instructions for additional reagent requirements.

Preparation of TMB Substrate

Number of Microelisa Strips	Volume of TMB Solution	Volume of Peroxide Solution
3	2 mL	2 mL
6	3 mL	3 mL
9	5 mL	5 mL
12	6 mL	6 mL

Number of Plates	Volume of TMB Solution	Volume of Peroxide Solution
1	6 mL	6 mL
2	12 mL	12 mL

The **TMB Substrate** is stable for 6 hours when held at room temperature and should be colorless when used. Record the preparation and expiration times. If it is noticeably blue in color, discard and prepare more **TMB Substrate** as required.

Note: **TMB Solution** and **TMB Substrate** should be protected from exposure to light. Avoid contact with metal or metal ions as it may result in unwanted blue color formation.

KIT STORAGE INSTRUCTIONS

Store kit reagents at 2-8°C. The expiration date of the kit is recorded on the kit label. Stability of kit reagents after reconstitution or dilution is listed in "REAGENT PREPARATION." Do not store frozen.

VioOne™ HIV Profile™ Strips

The re-sealable foil pouches should be brought to room temperature (15-30°C) before opening to prevent condensation. After the airtight foil pouch has been opened, any remaining Strips should be resealed in the foil pouch using the ziplock closure and stored at 2-8°C. The silica gel bag must not be removed. Stability of Strips resealed in the foil pouch after opening and stored at 2-8°C is 14 days.

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 citrate, sodium heparin, or EDTA (ethylenediaminetetraacetate) as anticoagulants may be used.

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.

Shipment:

Specimens to be shipped must be packaged in compliance with applicable regulations governing the transport of etiologic agents. Specimens may be shipped ambient, refrigerated (2-8°C), or frozen (-20°C or colder). Upon receipt, specimens should be stored at the recommended storage temperature described above.

VioOne™ HIV PROFILE™ TEST PROCEDURE

Materials provided

VioOne™ HIV Profile™ Strips
Sample Diluent
Negative Control Serum (Human)
HIV-1/2 Positive Control Serum (Human)
Conjugate
Conjugate Diluent
TMB Solution
Peroxide Solution
Wash Buffer Concentrate (provided separately as an accessory)

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%, 1000 µl ± 5%, and tips

Incubator

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

Microplate reader

Any microplate reader capable of transmitting light at 450 nm ± 5 nm with a linear absorbance range of 0 to 2.000.

Timer

Graduated cylinder, 50 ml and 1-2.5 L or equivalent

Reagents/Disposables

2N Sulfuric Acid
Purified Water, USP[7] or NCCLS Type I[7] reagent water, or equivalent
Stripholder with uncoated wells
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

Procedural notes

1. **Assay Strips**, **Conjugate, Negative Control**, and **Positive Control** used in an assay must be from the same master lot number. 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. **Assay 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. **Assay 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.
5. All reagents and specimens must be mixed well before use. The **HIV-1/2 Positive Control** and **Negative Control** may be vortexed before pipetting.
6. One microelisa strip containing 8 wells of **HIV-1/2 Positive Control** and one microelisa strip containing 8 wells of **Negative Control** must be included in each run.
7. If more than one stripholder is processed, ensure that all specified incubation times are met.
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 the **Microelisa Strips** 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 and specimens, use individual, disposable specimen tips to prevent carryover. 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 for that antigen.
12. Minimize opening the door of the incubator during the 37°C incubation time.
13. 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.
14. The aspiration/wash system should be flushed with copious amounts of water upon completion of the final wash of the assay. Refer to manufacturer's recommendations for the maintenance of the liquid handling system for automated microplate processors.

15. 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.
16. Do not return leftover reagents to their original bottles.
17. Do not touch the bottom exterior surface of the microwells. Fingerprints or scratches may interfere with the reading of microwells.
18. 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.
19. **Negative Control** or **HIV-1/2 Positive Control** values that are not within the expected range (refer to Quality Control section) may indicate a problem with technique, product, or instrumentation.
20. 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.
21. Bubbles in the Strip wells may cause inaccurate microwell readings. Care should be taken to ensure that no bubbles are present.
22. Use only properly calibrated equipment.

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 bottle. 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

23. Fit stripholder with the required number of **Assay Strips**. If less than twelve Strips are needed, use uncoated strips to complete the plate when using a 96-well washer.
24. To each strip (Controls and test specimens) pipet 80 µL of Sample Diluent to each well if using the *direct manual method* shown below.
25. Pipet 20 µL of each serum or plasma test specimen, or **Negative Control**, or **HIV-1/2 Positive Control** into each of 8 wells of a designated **Assay Strip** and repeatedly aspirate and dispense to mix while trying to minimize the formation of bubbles. Include one **Assay Strip** containing 8 wells of **Negative Control** and one **Assay Strip** containing 8 wells of **HIV-1/2 Positive Control** in each run. Include one **Assay Strip** containing 8 wells for each specimen tested.

    NOTE: It is suggested to pipet **HIV-1/2 Positive Control** to **column 1** of each plate and **Negative Control** to **column 2** of each plate.

Caution: Use a clean tip for adding specimen or **Controls** to each well when using the *Direct manual method* shown below. Do not pipet specimen into an empty well without **Sample Diluent**. Do not allow microelisa wells to dry once the assay has begun.

a) *Direct manual method*: Pipet 80 µL of Sample Diluent to each well of a designated **Assay Strip**. Pipet 20 µL of specimen or **Control** into each well of the designated **Assay Strip**. Repeatedly aspirate and dispense to mix while trying to minimize the formation of bubbles.

b) *Premixed manual method :* Pipet 200 µL specimen, **Negative Control**, or **Positive Control** into a clean test tube containing 800 µL **Sample Diluent**. Mix well but try to minimize bubble formation. Pipet 100 µL of the diluted specimen or **Control** into each well of the designated **Assay Strip**.

26. Cover the Strips with an adhesive plate sealer or equivalent. Incubate Strips at 37 ± 2°C for 60 ± 5 minutes.
27. Wash each well four times with Wash Solution (refer to "Wash procedure") using a soak cycle of at least 30-seconds.
28. Pipet 100 µL of **Conjugate Working Solution** into each well.

    Caution: Do not allow **Conjugate** to contaminate **TMB Substrate Solution**. If the same equipment is used to add both reagents, new disposable tips must be used.
29. Cover the Strips with an adhesive plate sealer or equivalent. Incubate at 37 ± 2°C for 30 ± 5 minutes.
30. Wash each well four times with Wash Solution (refer to "Wash procedure") using a soak cycle of at least 30-seconds.
9. Pipet 100 µL of **TMB Substrate** into each well. Do not mix or agitate. Do not cover the Strips.
10. Incubate at room temperature (15-30°C) for 30 ± 5 minutes.
11. Stop the reaction by adding 100 µL of **2N Sulfuric Acid** to each well (maintain the same sequence and time intervals used for **TMB Substrate** addition). **Plates should be read within 30 minutes.**
12. Blank the microelisa reader on air (without stripholder and Strips) and read the absorbance of the solution in each well at 450 nm ± 5 nm.

Qualification of Negative Control (NC) values:
Individual NC absorbances for each of eight wells are expected to be <0.200. Eliminate any values ≥0.200 and calculate the NC mean. Absorbance of remaining individual NC values are expected to be less than or equal to 1.7 multiplied by NC mean and greater than or equal to 0.5 multiplied by NC mean. Eliminate any outliers and recalculate the mean NC. If more than two NC values of eight total values are eliminated, the run is invalid and must be repeated.

CALCULATION OF CUTOFF VALUE

Calculate the cutoff value (COV) as follows:

COV = NCX x 2.5

Qualification of HIV-1/2 Positive Control (PC) values:
The individual PC S/CO values must meet expected results shown below. If the expected results are not met, the run is invalid and must be repeated.

Qualification of PC Values

Well	Solid Phase Antigen	S/CO
A	No viral antigens	< 1.0
B	HIV-1 *pol* gene product (p65)	> 3.0
C	HIV-1 reduced *env* gene product (gp160)	< 2.5
D	HIV-1 *env* gene product (gp160)	> 4.0
E	HIV-1 *env* gene product (Group M & O gp41)	> 4.0
F	HIV-1 *gag* gene product (p24)	> 4.0
G	NA*	NA
H	HIV-2 gp36	> 2.0

*Not Applicable except to calculate the Negative Control (NC) Mean.

If the S/CO value of any well does not match the criteria shown in the above table, the run is invalid and should be repeated.

Qualification of test specimens:
Any test specimens with an absorbance value greater than or equal to the cutoff value on Well A (no viral antigens), OR a reactive result in Well C and nonreactive result in Well D, OR a reactive result in Well C greater than a reactive result in Well D is invalid and must be repeated.

RESULTS

Calculations

Calculations must be made separately for each stripholder.

A specimen well is nonreactive for antibody if the absorbance is less than the cutoff value.

A specimen well is reactive for antibody if the absorbance is greater than or equal to the cutoff value.

Sample Calculations

Absorbance (example)

Well Designation	Solid Phase Antigen	NC Absorbance	PC Absorbance	PC S/CO
A	No viral antigens	0.086	0.094	0.62
B	HIV-1 *pol* gene product (p65)	0.063	1.388	9.07
C	HIV-1 reduced *env* gene product (gp160)	0.047	0.072	0.47
D	HIV-1 *env* gene product (gp160)	0.083	1.253	8.19
E	HIV-1 *env* gene product (Group M & O gp41)	0.061	0.987	6.45
F	HIV-1 *gag* gene product (p24)	0.048	1.166	7.62
G	NA*	0.065	NA	NA
H	HIV-2 gp36	0.051	0.781	5.10

*Not Applicable except to calculate the Negative Control (NC) Mean.

Acceptance Criteria
Eliminate any control absorbance values not meeting the following criteria:
NC < 0.200
NC ≤ 1.7 (NCx) or 0.104
NC ≥ 0.5 (NCx) or 0.031
PC acceptance as defined in **Qualification of PC S/CO Values** above.

For the above examples, none were eliminated.

Calculate Cutoff Value

NCX = 0.063

COV = NCX x 2.5

COV = 0.063 x 2.5 = 0.158

Recency Index (RI) Calculation

Only HIV-1 positive samples may be used to calculate recency index (RI) for estimation of an HIV-1 infection duration.

Recency Index (RI) = S/CO of Well C x S/CO of Well D

Recency Index (example)

Well Designation	Solid Phase Antigen	Sample 1 S/CO	Sample 2 S/CO	Sample 3 S/CO
A	No viral antigens	0.48	0.53	0.41
B	HIV-1 *pol* gene product (p65)	0.44	11.53	20.27
C	HIV-1 reduced *env* gene product (gp160)	0.32	0.67	1.94
D	HIV-1 *env* gene product (gp160)	0.38	16.56	20.95
E	HIV-1 *env* gene product (Group M & O gp41)	0.51	3.24	20.10
F	HIV-1 *gag* gene product (p24)	0.55	20.05	11.76
G	NA	NA	NA	NA
H	HIV-2 gp36	0.44	0.52	0.32
Infection Time Estimation	Recency Index (RI)	NA (Negative Sample)	11.10	40.64
	Interpretation		Recent HIV-1 Infection	Long Term HIV-1 Infection

INTERPRETATION OF ASSAY RESULTS FOR ESTIMATION OF HIV-1 INFECTION DURATION

1. Only confirmed HIV-1 positive samples may be used to estimate an HIV-1 infection duration
2. A recency index (RI) value of equal to or greater than18.00 indicates a long term HIV-1 infection (> 12 months); a RI value of less than 18.00 indicates a recent infection (≤ 12 months).

INTERPRETATION OF ASSAY RESULTS FOR CONFIRMATION OF AN HIV INFECTION

1. Specimen wells with absorbance values less than the cutoff value (S/CO < 1.0) are considered nonreactive for antibody**.**
2. Specimen wells with absorbance values greater than or equal to the cutoff value (S/CO ≥ 1.0) are considered reactive for antibody**.**
3. An HIV-1 infection is confirmed when the signal is equal to or above cutoff (S/CO ≥ 1.0) for any two or more of the wells coated with HIV-1 p65 (row B), gp160 (row D), gp41 (row E), and p24 (row F).
4. An HIV-2 infection is confirmed when the signal is equal to or above cutoff (S/CO ≥ 1.0) for the well coated with HIV-2 gp36 (row H).
5. **Table 1** below provides a guide for interpreting the test results of the VioOne™ HIV Profile™ Supplemental Assay
6. **Diagram 1**, below, shows examples of various antibody reactivity patterns that can be expected from testing serum or plasma samples with the VioOne™ HIV Profile™ Supplemental Assay.

Table 1: Results Interpretation Guide for Confirmation of an HIV Infection

Cat.	Test Results		Results Interpretation	
	HIV-1 Antigens	**HIV-2 Antigen**	**Interpretation**	**Symbol**
1	Nonreactive[1] for all HIV-1 Ags[2]	Nonreactive for HIV-2 Ag	**HIV Negative**	**NEG**
2	Reactive[1] for 1 HIV-1 Ag only	Nonreactive for HIV-2 Ag	**HIV-1 Indeterminate**	**HIV-1 IND**
3	Reactive for 2 or more HIV-1 Ags	Nonreactive for HIV-2 Ag	**HIV-1 Positive**	**HIV-1 POS**
4	Reactive for 1 or no HIV-1 Ag(s)	Reactive for HIV-2 Ag	**HIV-2 Positive**	**HIV-2 POS**
5	Reactive for 2 or more HIV-1 Ags	Reactive for HIV-2 Ag	**a. HIV-1 Positive with Reactivity to HIV-2 Antigen** (HIV-1 gp41 S/CO > HIV-2 gp36 S/CO) **b. HIV-2 Positive with Reactivity to HIV-1 Antigens** (HIV-1 gp41 S/CO ≤ HIV-2 gp36 S/CO)	**HIV-1 POS*[3]** **HIV-2 POS*[3]**

Interpretation Table legend:

1. Nonreactive: signal to cutoff ratio (S/CO) is less than 1.0; Reactive: S/CO ≥ 1.0
2. Ags: antigens
3. This test result does not exclude the possibility of an HIV-1 and HIV-2 coinfection (rare).

Diagram 1: Illustrative Examples of Possible Test Results

		Strip / Sample Number											
Well #	Coated Antigen	1	2	3	4	5	6	7	8	9	10	11	12
A	No Antigen	○	○	●	○	○	○	○	○	○	○	○	○
B	HIV-1 p65	●	●	○	●	●	●	●	○	○	○	○	●
C	HIV-1 gp160 (low Ag control)	●	○	○	●	○	●	○	○	○	○	○	●
D	HIV-1 gp160	●	●	●	○	●	●	○	○	○	○	○	●
E	HIV-1 gp41	●	●	●	●	○	○	○	○	○	○	○	●
F	HIV-1 p24	●	●	○	○	○	●	○	●	○	○	●	●
G	NA	○	○	○	○	○	○	○	○	○	○	○	○
H	HIV-2 gp36	○	○	○	○	○	○	○	○	●	●	●	●

This diagram illustrates various possible test results. The highlighted wells are those with S/CO greater than or equal to 1.0. Not all possible test results are included in this diagram.

In this example, samples 3 and 4 are invalid since the signal for the no antigen well for sample 3 is greater than the cutoff and the signal for the well undercoated with gp160 (Well C) is above the cutoff and the well coated with normal level of gp160 (Well D) is below cutoff for sample 4.

The test results for other samples are valid with the following interpretations: Samples 7 and 8 are HIV-1 indeterminate as there is only one HIV-1 antigen coated well with signal above the cutoff for each of these samples.

Samples 1, 2, 5 and 6 are confirmed for HIV-1 infection. Samples 9, 10, 11 are confirmed for HIV-2 infection. Since all wells coated for HIV-1 and HIV-2 antigens result in signal above the cutoff, sample 12 result is interpreted according to Category 5 a. or 5 b. in **Table 1**.

LIMITATIONS OF THE PROCEDURE

1. Serum or plasma derived from sodium citrate, sodium heparin, or EDTA (ethylenediaminetetraacetate) as anticoagulants may be used with HIV Profile™. Using other types of samples may not yield accurate results.
2. A HIV Profile™ test result that is INVALID should not be reported and the sample(s) should be retested.
3. A positive HIV Profile™ test result interpretation confirms the presence of specific antibodies to HIV-1 and/or HIV-2 in the sample. HIV and AIDS-related conditions are clinical syndromes caused by HIV-1 and HIV-2 and their diagnosis can only be established clinically.
4. False negative results may occur in individuals infected with HIV-1 and/or HIV-2 who are receiving highly active antiretroviral therapy (HAART).
5. A negative or indeterminate result does not preclude the possibility of exposure to HIV or infection with HIV. An antibody response to a recent exposure may take several months to reach detectable levels.
6. A person who has antibodies to HIV-1 or HIV-2 is presumed to be infected with the virus, however, 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.
7. Samples which meet the HIV-2 Positive criteria can show reactivity on one or more HIV-1 antigens. This profile that confirms an HIV-2 infection does not exclude the rare possibility of a coinfection.

8. Samples which meet both the HIV-1 and HIV-2 Positive criteria are interpreted as either (1) HIV-1 Positive with reactivity to HIV-2 antigen if HIV-1 gp41 S/CO > HIV-2 gp36 S/CO or (2) HIV-2 Positive with reactivity to HIV-1 antigens if HIV-1 gp41 S/CO ≤ HIV-2 gp36 S/CO. However, neither test result excludes the possibility of an HIV-1 and HIV-2 coinfection (rare).

PERFORMANCE CHARACTERISTICS OF THE ASSAY

Limit of Detection

The analytical sensitivity of the VioOne™ HIV Profile™ Supplemental Assay was compared to that of an FDA approved HIV-1/2 Supplemental assay by testing a panel of diluted HIV-1 and HIV-2 positive samples. Members of a study panel consisting of 4 HIV-1 and 4 HIV-2 samples were terminally diluted to undetectable levels of HIV antibodies in HIV antibody negative human serum. These samples were tested with the HIV Profile™ Assay. For comparison, the same samples were blinded and sent to a qualified third party laboratory for testing with an FDA approved HIV-1/2 supplemental assay. The study results are presented in **Table 2**.

Table 2: Summary of Test Results for Terminally Diluted Positive Samples

			VioOne™ HIV Profile™ Assay					
			HIV-1 (n=24)			HIV-2 (n=29)		
			POS	IND	NEG	POS	IND	NEG
HIV-1/2 Supplemental Assay (Comparator)	HIV-1	POS	0	0	0			
		IND	2	0	0			
		NEG	10	2	10			
	HIV-2	POS				12	0	0
		IND				5	0	1
		NEG				1	0	10

Of the 24 diluted HIV-1 samples, VioOne™ HIV Profile™ Supplemental Assay detected 12 as HIV-1 positive and two as HIV-1 indeterminate compared to none detected as HIV-1 positive and only two detected as HIV-1 indeterminate by an FDA approved HIV-1/2 supplemental assay (the comparator assay). HIV-1 samples used for the study were verified positive by the FDA approved Avioq HIV-1 Microelisa System. Of the 29 diluted HIV-2 samples, the VioOne™ HIV Profile™ Supplemental Assay detected 18 as HIV-2 positive and 11 as negative including one sample detected as HIV-2 indeterminate by the comparator. The comparator assay detected 12 as HIV-2 positive, 11 as negative including 1 detected as HIV-2 positive by the VioOne™HIV Profile™ Supplemental Assay, and 6 as HIV-2 or HIV indeterminate.

PERFORMANCE PANELS

HIV-1 Seroconversion Panels

A total of 77 samples from ten (10) commercially available seroconversion panels were tested with the HIV Profile™ Supplemental Assay. The test results were provided in **Table 3,** along with previously known test results from two other FDA approved supplemental assays. Since the comparator assays to generate the confirmatory test results with were performed several years ago for the first group of seroconversion panels, an additional 10 seroconversion panels were tested with both the VioOne™ HIV Profile Supplemental Assay and the Geenius™ HIV 1/2 Supplemental Assay. The results are shown in **Table 4**.

Table 3: Test Results of First Group of Seroconversion Panels

Panel			First Confirmed Positive Bleed (Days)		
Panel ID	Number of Panel Members	Collection Days	VioOne HIV Profile Supplemental Assay	Multispot Assay	Western Blot Test
1	10	0, 3, 8, 10, 15, 17, 26, 28, 33, 35	33	NA	33
2	5	2, 12, 24, 29, 31	12	12	NA
3	5	61, 63, 70, 72, 77	72	NA	70, 77*
4	6	0, 3, 7, 19, 24	24	NA	24
5	4	0, 24, 26, 33	26	24	NA
6	9	0, 2, 54, 117, 119, 124, 126, 129, 131	124	124	NA
7	6	0, 3, 7, 10, 18, 21	21	18	NA
8	3	0, 3, 8	8	8	NA
9	15	34, 41, 45, 50, 52, 57, 59, 64, 67, 71, 74, 78, 81, 88, 92	59	57	NA
10	14	48, 50, 56, 58, 65, 70, 72, 77, 79, 84, 86, 91, 93, 98	77	70	NA

*One Western Blot assay first confirmed positive panel member 70, a second first confirmed positive panel member 77.

Table 4: Test Results of Second Group of Seroconversion Panels

Panel			First Confirmed Positive Bleed (Days)	
Panel ID	Number of Panel Members	Collection Days	VioOne HIV Profile Supplemental Assay	Geenius™ HIV 1/2 Supplemental Assay
1	4	17, 38, 49, 51	49	None
2	3	59, 62, 67	67	67
3	3	3, 10, 49	49[a]	49
4	5	4, 9, 18, 21, 25	21	21
5	4	7, 10, 14	None[b]	None[b]
6	5	5, 8, 12, 16, 19	12	12
7	5	10, 14, 18, 21, 25	21	18
8	3	18, 25, 30	25	30
9	4	33, 35, 40, 42	40[a]	40
10	5	0, 4, 7, 25, 31	0	4

[a]One bleed earlier was detected as HIV-2 positive that was negative upon duplicate repeat testing.
[b]Both assays detected panel member 14 as HIV-1 Indeterminate.

HIV-1 Incidence / Prevalence Panel

All 15 members of the SeraCare PRB601 Incidence / Prevalence Panel, consisting of 7 known HIV-1 positive incidence (new infections) members and 8 known HIV-1 positive prevalence (long standing infections) members, were found to be HIV-1 antibody positive and HIV-2 antibody negative with the VioOne™ HIV Profile™ Supplemental Assay (**Table 5).**

Table 5: Test Results of SeraCare Incidence/Prevalence Panel (S/CO)

Panel Type	Well: Sample	A No Viral Ag	B p65	C gp160-D	D gp160	E gp41	F HIV-1 p24	G NA	H HIV-2 gp36	VioOne Profile
Incidence/ Prevalence	PRB601-1 (I)	0.370	7.812	0.533	12.273	4.612	20.311	NA	0.389	HIV-1 Pos
	PRB601-2 (I)	0.351	4.173	0.496	12.618	4.800	19.708	NA	0.358	HIV-1 Pos
	PRB601-3 (P)	0.408	17.468	1.274	23.373	19.997	20.988	NA	0.326	HIV-1 Pos
	PRB601-4 (P)	0.477	21.716	2.328	22.990	20.718	16.904	NA	0.370	HIV-1 Pos
	PRB601-5 (I)	0.414	2.886	0.376	6.431	1.995	17.707	NA	0.433	HIV-1 Pos
	PRB601-6 (P)	0.402	11.031	1.010	21.773	20.831	15.165	NA	0.389	HIV-1 Pos
	PRB601-7 (I)	0.370	1.826	0.703	13.961	5.001	13.666	NA	0.458	HIV-1 Pos
	PRB601-8 (P)	0.464	20.185	5.101	24.113	23.586	5.057	NA	0.602	HIV-1 Pos
	PRB601-9 (I)	0.376	10.121	0.646	18.811	10.987	15.736	NA	0.370	HIV-1 Pos
	PRB601-10 (P)	0.376	22.845	2.685	23.624	23.373	9.851	NA	0.489	HIV-1 Pos
	PRB601-11 (P)	0.301	18.334	1.779	19.267	18.899	14.797	NA	0.363	HIV-1 Pos
	PRB601-12 (I)	0.316	15.632	0.508	14.413	9.616	15.025	NA	0.311	HIV-1 Pos
	PRB601-13 (P)	0.441	15.149	2.002	19.532	19.184	8.469	NA	0.337	HIV-1 Pos
	PRB601-14 (I)	0.358	15.575	0.353	6.436	2.593	11.763	NA	0.301	HIV-1 Pos
	PRB601-15 (P)	0.347	17.857	0.628	15.772	12.727	10.637	NA	0.358	HIV-1 Pos

(I) - Incidence sample (recent infection)

(P) - Prevalence sample (long standing infection)

HIV-1 / HIV-2 Performance Panel

All 15 members of the SeraCare HIV-1 / HIV-2 Performance Panel 0800-0331, containing 7 HIV-1 positive, 7 HIV-2 positive, and 1 negative panel members were correctly identified and differentiated by the VioOne™ HIV Profile™ Supplemental Assay (**Table 6)**.

Table 6: Test Results of SeraCare HIV-1/HIV-2 Performance Panel (S/CO)

	Well	A	B	C	D	E	F	G	H	VioOne Profile
	Coated Antigen	No Viral Ag	p65	gp160-D	gp160	gp41	HIV-1 p24	NA	HIV-2 gp36	
Commercial Performance Panel Member	0800-0331-1	0.379	0.436	0.327	1.981	0.799	5.938	NA	0.332	HIV-1 Pos
	0800-0331-2	0.524	0.513	0.353	1.255	1.037	2.318	NA	0.441	HIV-1 Pos
	0800-0331-3	0.332	2.215	0.462	5.347	2.878	17.789	NA	0.415	HIV-1 Pos
	0800-0331-4	0.290	0.788	0.368	1.846	0.773	13.702	NA	0.363	HIV-1 Pos
	0800-0331-5	0.436	2.329	0.358	1.753	1.146	10.850	NA	0.358	HIV-1 Pos
	0800-0331-6	0.319	1.841	0.355	6.436	2.334	18.911	NA	0.283	HIV-1 Pos
	0800-0331-7	0.316	3.731	0.522	13.617	6.050	14.726	NA	0.286	HIV-1 Pos
	0800-0331-8	0.492	0.768	0.306	0.532	0.628	0.984	NA	7.818	HIV-2 Pos
	0800-0331-9	0.356	0.628	0.417	0.407	0.512	14.701	NA	7.843	HIV-2 Pos
	0800-0331-10	0.372	0.457	0.281	0.326	0.487	10.388	NA	7.361	HIV-2 Pos
	0800-0331-11	0.331	0.437	0.246	0.301	0.442	0.457	NA	8.857	HIV-2 Pos
	0800-0331-12	0.306	0.542	0.346	0.487	0.738	0.457	NA	6.969	HIV-2 Pos
	0800-0331-13	0.367	0.427	0.266	0.582	0.557	1.431	NA	7.074	HIV-2 Pos
	0800-0331-14	0.452	0.527	0.301	0.608	0.643	0.728	NA	8.375	HIV-2 Pos
	0800-0331-15	0.351	0.407	0.382	0.356	0.442	0.392	NA	0.291	Neg

HIV-1 Group M Clades

Nine (9) major HIV-1 Group M clades and 13 CRFs consisting of a total of 96 samples were tested in singlicate with the HIV Profile™ Supplemental Assay as shown in **Table 7**. All 96 Group M clade samples were tested positive for HIV-1 with the VioOne™ HIV Profile™ Supplemental Assay with 100% positivity rate (95% confidence interval: 96.2% - 100%). Of the 96 samples tested, all 96 were tested as HIV-1 positive; one of Clade C samples was also weakly reactive to HIV-2 gp36 antigen.

Table 7: HIV-1 Group M Clade Samples

HIV-1 Group M Clade	Number of Samples	VioOne™ HIV Profile™		
		NEG	IND	POS
A	10	0	0	10
B	10	0	0	10
C	10	0	0	10*
D	10	0	0	10
F	11	0	0	11
G	10	0	0	10
H	10	0	0	10
J	3	0	0	3
K	9	0	0	9
CRF01_AE	4	0	0	4
CRF02_AG	2			2
CRF06_cpx	2			2
CRF11_cpx	1			1
CRF14_BG	2			2
CRF18_cpx	1			1
CRF25_cpx	1			1
TOTAL	**96**	**0**	**0**	**96**

*1 Clade C sample tested HIV-1 positive with weak reactivity to the HIV-2 antigen.

Samples Repeatedly Reactive with an FDA Diagnostic or Screening HIV Assay but Confirmed Negative with a Supplemental Assay

A total of 100 samples that were repeatedly reactive (RR) with an FDA approved or licensed HIV-1/2 assay but were tested negative with a supplemental / confirmatory assay (*i.e.*, confirmed to be negative) were used in this study. Of these 100 repeatedly reactive samples, twenty-two (22) were confirmed negative by an HIV-1/2 Supplemental Assay, eighteen (18) were confirmed negative by an HIV-1/HIV-2 Rapid Confirmatory Test, and sixty (60) were confirmed negative by an IFA (Immunofluorescence Assay). The test results with the VioOne™ HIV Profile™ Supplemental Assay are summarized in **Table 8**.

Of these 100 samples, ninety-nine (99) samples were negative and one (1) sample was indeterminate with the VioOne™ HIV Profile™ Supplemental Assay. None of these samples were confirmed positive with the VioOne™ HIV Profile™ Supplemental Assay. Overall, there was a 99% (99/100) concordance for negativity confirmation and 1% (1/100) indeterminate rate with this group of samples.

Table 8: Test Results of Repeatedly Reactive and Confirmed Negative Samples

	Supplemental Assay Results	Number	VioOne™ HIV Profile™ Supplemental Assay		
			Negative	Indeterminate	Positive
Samples Repeatedly Reactive with a Diagnostic or Screening HIV Test	HIV-1/2 Supplemental Assay NEG	22	22	0	0
	HIV-1/HIV-2 Rapid Test NEG	18	18	0	0
	IFA NEG	60	59	1	0
	Total	**100**	**99 (99.0%)**	**1 (1.0%)**	**0**

Samples Repeatedly Reactive with an Approved Diagnostic or Screening HIV Assay but Indeterminate with a Supplemental Assay

A total of 107 samples which were repeatedly reactive (RR) with an FDA approved or licensed HIV-1/2 assay but indeterminate with an approved or licensed supplemental / confirmatory assay were tested with the VioOne™ HIV Profile™ Supplemental Assay. Of these 107 repeat reactive confirmed indeterminate samples, 8 were indeterminate with an approved HIV-1/2 Supplemental assay and 99 were indeterminate with an approved IFA (See **Table 9**).

Of the eight samples confirmed indeterminate with the HIV-1/2 Supplemental assay, one was indeterminate, three were negative, and four were HIV-1 antibody positive by the VioOne™ HIV Profile™ Supplemental Assay. Of the 99 samples confirmed indeterminate by IFA, ninety-two were negative, three were indeterminate, one four were antibody positive by the VioOne™ HIV Profile™ Supplemental Assay.

In addition, nineteen (19) samples which were repeatedly reactive with a diagnostic or screening HIV assay and confirmed positive with an IFA assay, but tested indeterminate with a Western Blot test, were tested with the VioOne™ HIV Profile™ Supplemental Assay. All of these 19 samples were confirmed to be positive by the VioOne™ HIV Profile™ Supplemental Assay.

Table 9: Test Results of Samples Repeatedly Reactive and Confirmed Indeterminate Samples

	Supplemental Assay Result	Number	VioOne™ HIV Profile™ Supplemental Assay		
			Negative	Indeterminate	Positive
Samples Repeatedly Reactive with a Diagnostic or Screening HIV Test	HIV-1/2 Supplemental Assay IND	8	3	1	4
	IFA IND	99	92	3	4
	Total	**107**	**95 (88.8%)**	**4 (3.7%)**	**8 (7.5%)**
	IFA POS, Western Blot IND	19	0	0	19

ANALYTICAL SPECIFICITY

Potential Interfering Factors

A panel of 130 retrospective samples from patients not known to be infected with HIV representing 13 categories of potentially interfering medical conditions unrelated to HIV infection were tested with the VioOne™ HIV Profile™ Supplemental Assay. Samples were tested unspiked (negative), spiked with an HIV-1 positive antibody, spiked with HIV-2 positive antibody, or spiked with both HIV-1 and HIV-2 positive antibodies.

Of the 130 potentially interfering factor samples tested unspiked, 125 (96.15%) were negative, 1 positive and 4 indeterminate with the VioOne™ HIV Profile™ Supplemental Assay (**Table 10)**.

Table 10: Test Results of Unspiked Samples with Potentially Interfering Factors

Potentially Interfering Factor	Number Tested	VioOne™ HIV Profile™		
		NEG	IND	POS
AutoImmune disease	10	10	0	0
Dialysis patients	10	10	0	0
EBV infection	10	10	0	0
HBsAg infection	10	10	0	0
HCV infection	10	10	0	0
High rheumatoid factor	10	10	0	0
Multiparous (pregnant) females	10	9	0	1[a]
Post influenza vaccine	10	9	1[b]	0
Yeast (Candida) reactive	10	7	3[c]	0
Vaccinia vaccine samples	10	10	0	0
HTLV-I/II antibody positive	10	10	0	0
Multiple transfusions	10	10	0	0
Hemophilia	10	0	0	0
TOTAL	**130**	**125 (96.15%)**	**4 (3.08%)**	**1 (0.77%)**

[a]HIV-2 positive that was negative on duplicate repeat testing.

[b]HIV-1 indeterminate that was negative on repeat testing in singlicate due to volume limitation.

[c]Of these three HIV-1 indeterminate samples, one was indeterminate upon repeat testing in duplicate, one was negative upon repeat testing in duplicate and, one was invalid for repeat testing.

Of the 130 potentially interfering factor samples spiked with HIV-1 positive antibody, all were positive for HIV-1 with the VioOne™ HIV Profile™ Supplemental Assay. One specimen from an autoimmune disease patient also showed borderline reactivity with HIV-2 gp36. Results from HIV-1 positive antibody spiked samples are shown in **Table 11**.

Table 11: Test Results of Potentially Interfering Factors Spiked HIV-1 Antibody

Potential Interfering Factor	Number Tested	VioOne™ HIV Profile™			
		NEG	IND	HIV-1 POS	HIV-2 POS
AutoImmune disease	10	0	0	10	1*
Dialysis patients	10	0	0	10	0
EBV infection	10	0	0	10	0
HBsAg infection	10	0	0	10	0
HCV infection	10	0	0	10	0
High rheumatoid factor	10	0	0	10	0
Multiparous (pregnant) females	10	0	0	10	0
Post influenza vaccine	10	0	0	10	0
Yeast (Candida) reactive	10	0	0	10	0
Vaccinia vaccine samples	10	0	0	10	0
HTLV-I/II antibody positive	10	0	0	10	0
Multiple transfusions	10	0	0	10	0
Hemophilia	10	0	0	10	0
TOTAL	**130**	**0**	**0**	**130 (100.0%)**	**1 (0.77%)**

*1 of 10 samples was HIV-1 positive and HIV-2 gp36 reactive.

Of the 130 potentially interfering factor samples spiked with HIV-2 positive antibody, all were positive for HIV-2 with the VioOne™ HIV Profile™ Assay. Five (5) of these specimens also tested positive for HIV-1 as well with the VioOne™ HIV Profile™ Supplemental Assay. Of these 5 HIV-1 positive samples, four (4) were confirmed positive with Western Blot and the remaining one was confirmed positive with an HIV 1/2 Supplemental Assay (**Table 12**); thus, all these five samples were originally positive for HIV-1 antibodies.

Table 12: Test Results of Potentially Interfering Factors Spiked with HIV-2 Antibody

Potential Interfering Factor	Number Tested	VioOne™ HIV Profile™			
		NEG	IND	HIV-1 POS	HIV-2 POS
AutoImmune disease	10	0	0	0	10
Dialysis patients	10	0	0	1[a]	10
EBV infection	10	0	0	0	10
HBsAg infection	10	0	0	0	10
HCV infection	10	0	0	3[b]	10
High rheumatoid factor	10	0	0	0	10
Multiparous (pregnant) females	10	0	0	0	10
Post influenza vaccine	10	0	0	0	10
Yeast (Candida) infection	10	0	0	1[b]	10
Vaccinia vaccine samples	10	0	0	0	10
HTLV-I/II antibody positive	10	0	0	0	10
Multiple transfusions	10	0	0	0	10
Hemophilia	10	0	0	0	10
TOTAL	**130**	**0**	**0**	**5 (3.85%)**	**130 (100%)**

[a]Confirmed positive by an HIV 1/2 Supplemental Assay
[b]Confirmed positive by a Western Blot Test

Of the 130 potentially interfering factor samples spiked with a blend of HIV-1 and HIV-2 antibodies, all were tested positive for both HIV-1 and HIV-2 with the VioOne™ HIV Profile™ Supplemental Assay (**Table 13)**.

Table 13: Results of Potentially Interfering Factors Spiked with HIV-1 and HIV-2 Antibody

Potential Interfering Factor	Number Tested	VioOne™ HIV Profile™		
		NEG	HIV-1 POS	HIV-2 POS
AutoImmune disease	10	0	10	10
Dialysis patients	10	0	10	10
EBV infection	10	0	10	10
HBsAg infection	10	0	10	10
HCV infection	10	0	10	10
High rheumatoid factor	10	0	10	10
Multiparous (pregnant) females	10	0	10	10
Post influenza vaccine	10	0	10	10
Yeast (Candida) infection	10	0	10	10
Vaccinia vaccine samples	10	0	10	10
HTLV-I/II antibody positive	10	0	10	10
Multiple transfusions	10	0	10	10
Hemophilia	10	0	10	10
TOTAL	**130**	**0**	**130 (100%)**	**130 (100%)**

To assess whether an elevated biotin concentration in a sample would have an adverse effect on the test results of the VioOne™ HIV Profile™ Supplemental Assay, an HIV negative human serum sample was spiked with 3600 ng/mL of exogenous biotin, which is three times the therapeutic level[8]. The HIV negative sample with or without spiked biotin was tested as unspiked or spiked with HIV-1 positive, HIV-2 positive, or HIV-1/HIV-2 antibody positive samples.

As shown by the VioOne™ HIV Profile™ Supplemental Assay S/CO results in **Table 14**, the human serum sample containing 3600 ng/mL of biotin performed similarly to the same sample without added biotin, regardless whether the samples were spiked or unspiked with HIV antibody (HIV-1 antibody, HIV-2 antibody, or a blend of HIV-1 & HIV-2 antibodies). Therefore, a level of biotin three times the highest biotin level that may be seen in clinical practice did not interfere with the performance of the VioOne™ HIV Profile™ Supplemental Assay.

Table 14: Test Results (S/CO) of Samples with and without an Elevated Level of Biotin

		Unspiked (Neg)		HIV-1 Spike		HIV-2 Spike		HIV-1/HIV-2 Spike	
	Biotin (3600 ng/mL)	No	Yes	No	Yes	No	Yes	No	Yes
Coated Antigen	No Antigen	0.289	0.307	0.349	0.397	0.397	0.361	0.391	0.379
	HIV-1 p65	0.349	0.325	7.591	8.144	0.722	0.650	3.435	3.146
	HIV-1 gp160-D	0.325	0.277	0.445	0.493	0.433	0.283	0.427	0.391
	HIV-1 gp160	0.319	0.307	7.789	8.716	0.451	0.343	5.913	6.105
	HIV-1 gp41/O	0.523	0.451	4.409	5.347	0.854	0.535	2.683	2.129
	HIV-1 p24	0.349	0.277	2.460	2.767	0.746	0.535	5.143	4.908
	NA	NA	NA	NA	NA	NA	NA	NA	NA
	HIV-2 gp36	0.343	0.355	0.331	0.289	5.672	5.780	5.678	5.901

Cross Reactivity Study

In a separate cross-reactivity study, a panel of 47 potentially cross-reactive samples representing nine different disease states was tested with the VioOne™ HIV Profile™ Supplemental Assay. All 47 samples were tested negative with the VioOne™ HIV Profile™ Supplemental Assay, indicating that the samples from these disease states did not result in cross reactivity in the VioOne™ HIV Profile™ Supplemental Assay (**Table 15**).

Table 15: Test Results on Additional Potentially Cross Reactive Samples

Disease State Samples	Number Tested	VioOne™ HIV Profile™		
		NEG	IND	POS
Cirrhosis	5	5	0	0
Hepatitis A	7	7	0	0
Cancer	5	5	0	0
HSV IgG	5	5	0	0
Malaria: P. falciparum	5	5	0	0
Rubella IgG	5	5	0	0
Syphilis	5	5	0	0
Toxoplasmosis IgG	5	5	0	0
CMV IgG	5	5	0	0
TOTAL	**47**	**47 (100%)**	**0**	**0**

Commercially available samples containing various levels of bilirubin, lipid (triglycerides), hemoglobin, or HAMA (human anti-mouse antibody) were tested with the VioOne™ HIV Profile™ Supplemental Assay. The test results showed that these substances did not cause cross reactivity in the VioOne™ HIV Profile™ Supplemental Assay (**Table 16**).

Table 16: Test Results on Additional Potentially Cross Reactive Samples

Panel Member Samples		**Number Tested**	**VioOne™ HIV Profile™**		
			NEG	**IND**	**POS**
Total Bilirubin	0.20 mg/dL	1	1	0	0
	2.00 mg/dL	1	1	0	0
	4.00 mg/dL	1	1	0	0
	6.70 mg/dL	1	1	0	0
	11.43 mg/dL	1	1	0	0
Lipemia - Triglycerides	150 mg/dL	1	1	0	0
	272 mg/dL	1	1	0	0
	379 mg/dL	1	1	0	0
	1013 mg/dL	1	1	0	0
	2375 mg/dL	1	1	0	0
Hemoglobin	Normal	1	1	0	0
	140 mg/dL	1	1	0	0
	275 mg/dL	1	1	0	0
	550 mg/dL	1	1	0	0
	1100 mg/dL	1	1	0	0
HAMA (Human anti mouse antibody)	Negative	1	1	0	0
	Negative	1	1	0	0
	4.0 ng/mL	1	1	0	0
	4.7 ng/mL	1	1	0	0
	7.2 ng/mL	1	1	0	0
	9.6 ng/mL	1	1	0	0
	13.0 ng/mL	1	1	0	0
	27.1 ng/mL	1	0	1*	0
	30.0 ng/mL	1	1	0	0
	38.8 ng/mL	1	1	0	0
	52.7 ng/mL	1	1	0	0
	74.0 ng/mL	1	1	0	0
TOTAL		**27**	**(26/27) 96.30%**	**(1/27) 3.70%**	**(0/27) 0.00%**

*HIV-1 Indeterminate that was negative upon repeat testing in duplicate.

REPRODUCIBILITY

A reproducibility panel consisting of 7 panel members with various antibody titers and the two kit controls were used in this study (**Table 17**). The 7 member reproducibility panel included a normal negative (R1), a high background negative (R2), an HIV-1 sample with low titer antibodies (R3), an HIV-1 sample with moderate levels of antibody (R4), an HIV-2 sample with moderate antibody titer (R5), an HIV-2 sample with high titer (R6), and a sample consisting of both HIV-1 and HIV-2 positive antibody (R7). The two kit controls (Kit PC and Kit NC) were also tested with the study panel members.

Each panel member and kit controls were tested in each of the three study sites 3 times per day with each of the three validation lots over a period of 5 days. A total of 135 replicates were tested for each panel member and controls (3 runs per day x 3 validation lots x 1 technician x 3 sites x 5 days). Since each panel member generated 8 test results from the 8 wells in a strip, there were a total of 1080 test results for each panel member and kit control.

Total percent (%) agreement of the VioOne™ HIV Profile™ Supplemental Assay test results compared to the expected results and 95% confidence interval of the percent agreement were determined for each study panel member and kit control. The results are shown in **Table 17**. This study demonstrated that the VioOne™ HIV Profile™ Supplemental Assay is highly reproducible.

Table 17: Test Results from the Reproducibility Study

Sample			Test Results			
Code Name	**Description**	**Expected Results**	**# of Replicates**	**# of Correct Test Results**	**% Agreement**	**95% CI**
Kit PC	HIV-1/HIV-2 Antibody Positive	HIV-1 / HIV-2 POS	135	135	100%	97.30% - 100%
Kit NC	HIV-1/HIV-2 Antibody Negative	NEG	135	135	100%	97.30% - 100%
R1	HIV-1/HIV-2 Antibody Negative	NEG	135	134	99.26%	95.94% - 99.98%
R2	HIV-1/HIV-2 Antibody Negative (high background)	NEG	135	132	97.78%	93.64% - 99.54%
R3	HIV-1 Low Antibody Positive	HIV-1 POS	135	133	98.52%	94.75% - 99.82%
R4	HIV-1 Moderate/High Antibody Positive	HIV-1 POS	135	134	99.26%	95.94% - 99.98%
R5	HIV-2 Moderate Antibody Positive	HIV-2 POS	135	135	100%	97.30% - 100%
R6	HIV-2 High Antibody Positive	HIV-2 POS	135	135	100%	97.30% - 100%
R7	HIV-1/HIV-2 Low Antibody Positive	HIV-1 / HIV-2 POS	135	135	100%	97.30% - 100%
Overall Agreement (including Kit Controls)			**1215**	**1208**	**99.42%**	**98.82%-99.77%**

CLINICAL SPECIFICITY

A total of 599 serum and plasma, including 20 serum pediatric samples, were collected from individuals at low risk for HIV infection and tested by the VioOne™ HIV Profile™ Supplemental Assay. Results are shown in **Table 18**. The overall clinical specificity for the VioOne™ HIV Profile™ in the low risk population was 98.16% (95% CI: 96.74% - 99.08%).

Table 18: Specificity of VioOne™ HIV Profile™ Assay in a Low Risk Population

Sample Type	Number	Negative	Indeterminate	Positive
Serum	279[a]	272	5[b]	2[d]
Pediatric Serum	20	20	0	0
Plasma	300	296	3[c]	1[e]
TOTAL	**599**	**588 (98.16%)**	**8 (1.36%)**	**3 (0.50%)**

[a]One sample was repeatedly invalid and excluded from analysis.
[b]All 5 samples were HIV-1 indeterminate and negative upon repeat testing in duplicate.
[c]All 3 samples were HIV-1 indeterminate and negative upon repeat testing in duplicate.
[d]Of the 2 samples, 1 was HIV-1 positive and 1 was HIV-2 positive and both were negative upon repeat testing in duplicate.
[e]Sample was HIV-2 positive and negative upon repeat testing in duplicate.

CLINICAL SENSITIVITY

A total of 744 samples collected from known HIV positive/AIDS patients were tested by the VioOne™ HIV Profile™ Supplemental Assay. Results are shown in **Table 19**. One sample from an HIV-1 positive patient was negative by initial and repeat testing with the VioOne™ HIV Profile™ Supplemental Assay. The overall clinical sensitivity for HIV Profile™ was 99.46% (95% CI: 98.62% – 99.85%).

Table 19: Sensitivity of VioOne™ HIV Profile™ Assay in Known HIV Positive / AIDS Patients

Sample Type	Number	Positive	Indeterminate	Negative
HIV-1 Positive	400	398[a]	1[b]	1
HIV-2 Positive	202	202[c]	0	0
HIV-1/HIV-2 Coinfection	10	10[d]	0	0
HIV-1 Group O	15	15	0	0
AIDS	50	50[e]	0	0
HIV-1 Positive Pediatrics	40	38[f]	2	0
HIV-1 Positive Pregnant Females	27	27	0	0
TOTAL	**744**	**740 (99.46%)**	**3 (0.40%)**	**1 (0.13%)**

[a]One sample was interpreted as HIV-1 positive with reactivity to HIV-2 antigen.

[b]HIV-1 indeterminate and HIV-1 upon repeat testing in duplicate.

[c]Fifty-four of 202 samples were interpreted as HIV-2 positive with reactivity to HIV-1 antigens. One (1) HIV-2 sample was interpreted as HIV-1 positive with reactivity to HIV-2 antigen. All other samples were HIV-2 positive.

[d]Ten samples were HIV-2 positive and 6 of 10 were interpreted as HIV-2 positive with reactivity to HIV-1 antigens.

[e]Four samples were interpreted as HIV-1 positive with reactivity to HIV-2 antigen.

[f]One sample was interpreted as HIV-1 positive with reactivity to HIV-2 antigen.

ESTIMATION OF HIV INFECTION TIME

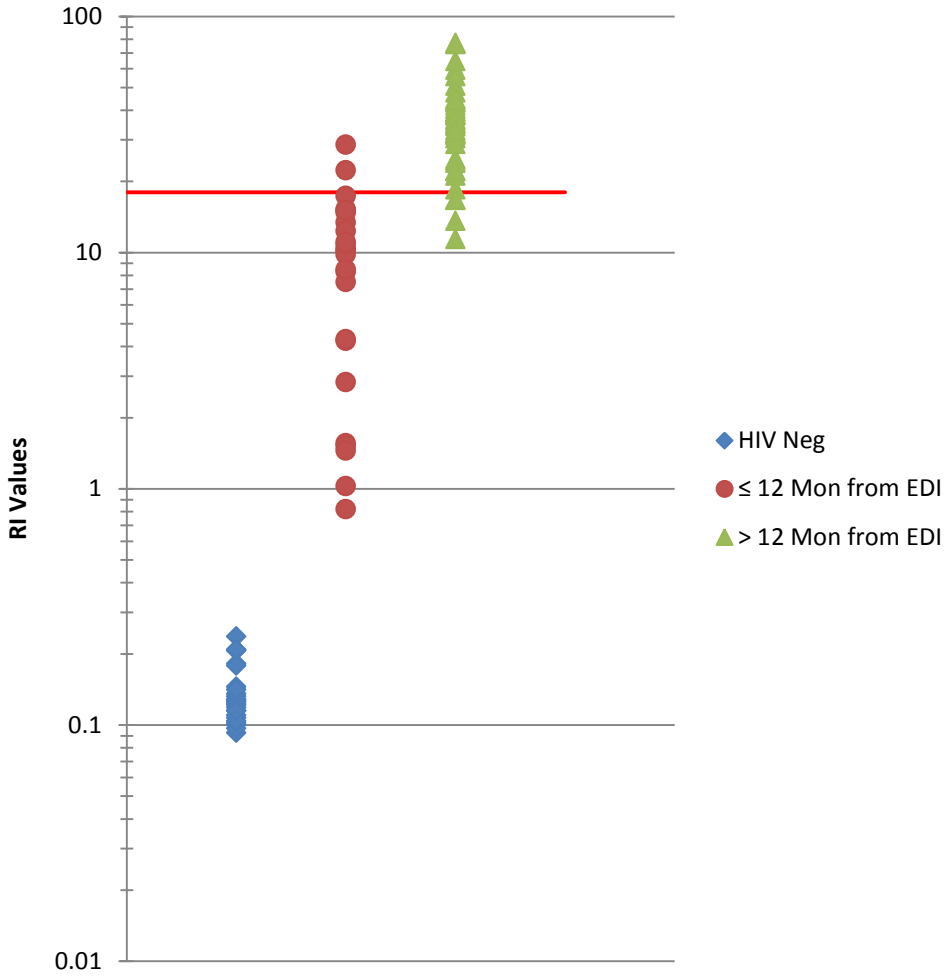
S/CO from two solid phase wells, one coated with normal (Well D) and the other coated with reduced concentrations (Well C) of HIV-1 gp160, was used to calculate a Recency Index (RI) for each sample. A Recency Index (RI) value is calculated as follows:

Recency Index (RI) = S/CO of Well C x S/CO of Well D

The cutoff RI value is 18.00. A recency index (RI) value of equal to or greater than18.00 indicates a long term HIV-1 infection (> 12 months); a RI value of less than 18.00 indicates a recent infection (≤ 12 months).

A total of 91 samples included in the HIV Recency Biomarker Screening Panel (HRBS), provided by the Consortium for Evaluation and Performance of HIV Incidence Assays (CEPHIA), were tested by the VioOne™ HIV Profile™ Assay. The HRBS panel consisted of 25 plasma samples from HIV negative individuals, 24 plasma samples from patients ≤12 months from estimated date of infection (EDI), and 42 plasma samples from patients ≥12 months from EDI. Test results are shown in **Fig 1**.

Fig 1: RI Values for Samples in CEPHIA HRBS Panel



The RI values ranged from 0.09 – 0.24 for samples from HIV negative individuals. In contrast, the RI values ranged from 0.82 – 28.62 for samples from patients ≤12 months from EDI and 11.42 – 77.27 for samples from patients ≥12 months from EDI. **Table 20** shows the numbers of samples, the average RI value from each patient population included in the CEPHIA HRBS panel, the number of samples from each patient population that were correctly classified, and the accuracy of the classification. Based on the use of an RI threshold value of 18.00, the samples included in the CEPHIA HRBS panel from patients ≤12 months from EDI could be distinguished from those samples from patients >12 months from EDI with an accuracy of at least 90%.

Table 20: Average RI of Sample Populations included in the CEPHIA HRBS Panel

Specimen Population	n	Average RI	Correct Classification	Percent Correct Classification
HIV Negative	25	0.14	25	100%
≤12 Months from EDI	24	9.7	22	92%
>12 Months from EDI	42	37.4	38	90%

This demonstrates that the VioOne™ HIV Profile™ Supplemental Assay can be used to differentiate a recent infection from a longstanding infection, which in turn may be used to estimate HIV-1 incidence in a population.

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AVAILABILITY

VioOne™ HIV Profile™ Supplemental Assay

24-Test Kit (2 X 96 well plates)	REF Product number 700024
Wash Buffer Concentrate (500 mL bottle)	REF Product number 759879
Wash Buffer Concentrate (4X500 mL bottle)	REF Product number 759880

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March 2019