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 confirmation and differentiation of antibodies directed to HIV-1 and HIV-2 in human serum or plasma

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 VioOne™ HIV Profile™ Supplemental Assay 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.
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.
4 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 Thermo Fisher

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. The rehydrated Conjugate Concentrate cnnot be stored and must be discarded after use..

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).

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.

Kits exposed to extreme shipping conditions between -20°C and 45°C for 50 hours remain stable up to 7 months after receipt when kit reagents are stored at 2-8°C and Wash Buffer Concentrate is stored at room temperature.

HIV Profile™ Supplemental Assay 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

The colored or colorless solutions should be clear and visually free of particulate matter. 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

Serum or plasma derived from sodium citrate, CPD (citrate phosphate dextrose), 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 seven (7) days. For long-term storage, specimens should be frozen at –20°C for up to twelve (12) months. 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 refrigerated (2-8°C), or frozen (-20°C or colder). Upon receipt, specimens should be stored at the recommended storage temperature described above.

HIV PROFILE™ SUPPLEMENTAL ASSAY 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)
Plate sealers - adhesive

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 (approximately 0.3 ml) with Wash Solution, unless otherwise validated. 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

1.‘ 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.
2. To each strip (Controls and test specimens) pipet 80 µL of Sample Diluent to each well if using the *direct manual method* shown below.
3. 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**.

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

   Caution: Do not allow **Conjugate** to contaminate **TMB Substrate.** If the same equipment is used to add both reagents, new disposable tips must be used.
7. Cover the Strips with an adhesive plate sealer or equivalent. Incubate at 37 ± 2°C for 30 ± 5 minutes.
8. 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:

Step 1:

Individual NC absorbances for each of eight wells are expected to be <0.200. Eliminate any values ≥0.200 and calculate the NC mean (NCX). Absorbance of remaining individual NC values are expected to be less than or equal to 1.7 multiplied by NCX and greater than or equal to 0.5 multiplied by NCX.

Step 2:

Eliminate any additional NC values that are outside of the range 0.5 ≤ NCX ≤ 1.7 calculated in Step 1. Recalculate the NCX. If more than two NC values of eight total NC values are eliminated, the run is invalid and must be repeated.

Step 3:

If the NCX is 0.100 absorbance or greater, the run is invalid and the HIV Profile™ Supplemental Assay 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) for control assay	< 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) for assay control	0.047	0.072	0.47
D	HIV-1 *env* gene product (gp160)	0.213	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*	0.168	NA	NA
H	HIV-2 gp36	0.051	0.781	5.10

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

NC Acceptance Criteria

Step 1: Eliminate NC values ≥ 0.200 absorbance, therefore, eliminate 0.213.
Calculate NCX (mean of NC values other than 0.213); NCX = 0.075
Calculate NC value acceptable range, which must fall between 0.5 NCX and 1.7 NCX:
0.5 NCX = 0.075 x 0.5 = 0.037
1.7 NCX = 0.075 x 1.7 = 0.127
The acceptable NC range is 0.037 – 0.127

Step 2: Eliminate NC values outside 0.037 - 0.127; therefore, eliminate 0.168.
The six remaining NC values are acceptable.
Recalculate NCX using the remaining 6 NC values: NCX = 0.059

Step 3: 0.059 <0.100; therefore, the NC is acceptable.

Calculate Cutoff Value

NCX = 0.059
COV = NCX x 2.5
COV = 0.059 x 2.5 = 0.148

PC acceptance is defined in **Qualification of PC S/CO valves** above:
All PC values acceptable

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

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).

The diagram below shows examples of various antibody reactivity patterns that can be expected from testing serum or plasma samples.

		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.

In this example, samples 3 and 4 are invalid. For sample 3, the signal for the no antigen well is greater than the cutoff. For sample 4, 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 the cutoff.

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. For *in vitro* diagnostic use.
2. For prescription use only
3. This device is not intended for use as a first line diagnostic test or for screening donors of blood, blood products, or human cells or tissues or cellular and tissue-based products (HCT/Ps).
4. Only serum or plasma derived from sodium citrate, CPD (citrate phosphate dextrose), heparin, or EDTA (ethylenediaminetetraacetate) as anticoagulants may be used. Using other types of samples may not yield accurate results.
5. The HIV Profile™ Supplemental Assay must be used in accordance with the instructions for use in the package insert to obtain accurate results.
6. Test results are not intended as an initial diagnostic test or for monitoring individuals who are undergoing treatment for HIV infection.
7. All test results should be interpreted in conjunction with the individual's clinical presentation, history, and other laboratory results.

8. A test result that is INVALID should not be reported and the sample(s) should be retested.
9. A positive 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.
10. 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. A comprehensive risk history and clinical judgement should be considered before concluding that an individual is not infected with HIV.
11. False negative results may be obtained under the following conditions:
    a. In individuals infected with HIV-1 and/or HIV-2 who are receiving medication for treatment for HIV infection (ART) or prevention of infection (PrEP or PEP).
    b. 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 weeks to months to reach detectable levels. A follow-up HIV-1 NAT test should be considered when results of an HIV donor screening assay are reactive and the HIV Profile™ Supplemental Assay result is nonreactive or indeterminate. It is recommended that testing be repeated on a specimen freshly drawn after 2–4 weeks.
    c. Immunosuppressed or immunocompromised individuals infected with HIV-1 and/or HIV-2 may not produce antibodies to the virus.
    d. Antibodies to a variant strain of HIV-1 and/or HIV-2 in the patient that do not react with specific antigens utilized in the assay configuration.

Results interpretation limitations:

1. HIV-2 positive samples exhibited a high degree of cross-reactivity to HIV-1 antigens in the HIV Profile™ Supplemental Assay (see Table 18, footnote a).
2. Results that meet the HIV-2 Positive criteria can show reactivity on one or more HIV-1 antigens. A result that confirms an HIV-2 infection does not exclude the rare possibility of a co-infection.
3. Results that meet both the HIV-1 and HIV-2 Positive criteria are interpreted as either HIV-1 Positive with reactivity to HIV-2 antigen or HIV-2 Positive with reactivity to HIV-1 antigens. However, neither test result excludes the possibility of an HIV-1 and HIV-2 coinfection (rare).
4. An Indeterminate interpretation does not exclude the possibility of early seroconversion of the test subject or a cross-reactivity with other retroviruses. The homology between HIV-1 and HIV-2 viruses can lead to cross-reactivities between anti-HIV-1 and anti-HIV-2 antibodies.

PERFORMANCE CHARACTERISTICS OF THE ASSAY

Analytical Sensitivity

The analytical sensitivity of the VioOne™ HIV Profile™ Supplemental Assay was evaluated 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 confirmed positive samples were terminally diluted to undetectable levels of HIV antibodies in HIV antibody negative human serum and tested. 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

			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	11	3	8			
	HIV-2	POS				12	0	0
		IND				5	0	1
		NEG				2	0	9

Of the 24 diluted HIV-1 samples, the HIV Profile™ Supplemental Assay detected 13 as HIV-1 positive and three 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). Of the 29 diluted HIV-2 samples, the HIV Profile™ Supplemental Assay detected 19 as HIV-2 positive and 10 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 2 detected as HIV-2 positive by the HIV Profile™ Supplemental Assay, and 6 as HIV-2 or HIV indeterminate.

PERFORMANCE PANELS

HIV-1 Seroconversion Panels

A total of 88 samples from twenty (20) commercially available seroconversion panels were tested with the HIV Profile™ Supplemental Assay and an FDA approved Supplemental Assay by a qualified third party laboratory. The test results are shown in **Table 3.**

Table 3: Test Results of First Group of Seroconversion Panels

Panel			First Confirmed Positive Bleed (Day)	
Panel ID	**Number of Panel Members**	**Collection Days**	**HIV Profile™ Supplemental Assay**	**HIV 1/2 Supplemental Assay**
1	4	17, 38, 49, 51	49	49 (Ind*)
2	3	59, 62, 67	67 (Ind)	67
3	3	3,10, 49	49	49
4	3	7, 10 14	14 (Ind)	14 (Ind)
5	5	10, 14, 18, 21, 25	21	18
6	3	18, 25, 30	30	30
7	4	33, 35, 40, 42	40	40
8	3	13, 15, 20	20 (Ind)	20 (Ind)
9	3	17, 19, 24	24	24
10	4	0, 27, 30, 34	34 (Ind)	34 (Ind)
11	10	52, 57, 59, 64, 67, 71, 74, 78, 81 88,	57	52
12	10	56, 58, 65, 70, 72, 77, 79, 84, 86, 91	65	58
13	4	63, 70, 72, 77	72	77
14	3	0, 3, 8	8	8
15	3	10, 18, 21	21	18
16	4	0, 24, 26, 33	26	26
17	5	117, 119, 124, 126, 129	124	126
18	5	28, 30, 36, 45, 53	45	36
19	4	26, 28, 33, 35	33	33
20	5	2, 12, 24, 29, 31	12	24

*Indeterminate

Of 88 samples included in the 20 Seroconversion panels, 70 (80%) were interpreted as the same between the HIV Profile™ Supplemental Assay and an FDA approved HIV 1/2 supplemental assay (35 POS, 7 IND, & 28 NEG). There were a total of 42 samples interpreted as POS by the HIV Profile™ Supplemental Assay vs 41 POS by an FDA approved HIV 1/2 supplemental assay. Each assay interpreted 15 samples as IND while the HIV Profile™ Supplemental Assay interpreted 31 samples as NEG compared to 32 by an FDA approved HIV 1/2 supplemental assay.

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 4**

Table 4: 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)	0.553	13.582	0.745	23.580	4.807	25.408		0.516	HIV-1 Pos
	PRB601-2 (I)	0.494	6.968	0.745	22.769	3.687	25.194		0.450	HIV-1 Pos
	PRB601-3 (P)	0.413	22.577	2.190	28.129	23.830	25.902		0.487	HIV-1 Pos
	PRB601-4 (P)	0.538	27.760	2.485	28.490	24.347	22.599		0.516	HIV-1 Pos
	PRB601-5 (I)	0.568	5.132	0.465	16.951	2.514	22.960		0.383	HIV-1 Pos
	PRB601-6 (P)	0.597	16.354	1.519	28.181	24.398	21.434		0.560	HIV-1 Pos
	PRB601-7 (I)	0.450	2.492	0.826	25.504	6.304	19.952		0.369	HIV-1 Pos
	PRB601-8 (P)	0.442	26.168	5.677	28.660	27.185	9.386		0.413	HIV-1 Pos
	PRB601-9 (I)	0.406	16.413	1.010	27.185	10.890	21.965		0.347	HIV-1 Pos
	PRB601-10 (P)	0.531	27.679	3.281	28.719	27.082	13.242		0.391	HIV-1 Pos
	PRB601-11 (P)	0.513	25.502	2.518	26.289	25.579	16.506		0.429	HIV-1 Pos
	PRB601-12 (I)	0.492	23.089	0.886	24.629	12.118	18.764		0.394	HIV-1 Pos
	PRB601-13 (P)	0.626	22.133	3.854	26.247	25.326	11.998		0.689	HIV-1 Pos
	PRB601-14 (I)	0.654	24.250	0.556	17.196	3.130	14.270		0.605	HIV-1 Pos
	PRB601-15 (P)	0.647	25.804	1.639	24.665	18.342	14.024		0.527	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 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
HIV-1/HIV-2 Performance	0800-0331-1	0.549	0.745	0.359	6.238	0.816	5.894		0.443	HIV-1 Pos
	0800-0331-2	0.682	1.280	0.464	3.024	0.668	3.116		0.380	HIV-1 Pos
	0800-0331-3	0.443	4.283	0.436	13.778	3.158	21.148		0.366	HIV-1 Pos
	0800-0331-4	0.436	1.871	0.429	5.788	0.844	16.197		0.380	HIV-1 Pos
	0800-0331-5	0.513	3.974	0.401	4.846	0.992	13.911		0.394	HIV-1 Pos
	0800-0331-6	0.429	3.767	0.531	15.331	2.138	24.596		0.444	HIV-1 Pos
	0800-0331-7	0.385	7.280	0.851	25.040	8.058	22.022		0.364	HIV-1 Pos
	0800-0331-8	0.451	0.727	0.400	1.251	0.684	1.367		15.745	HIV-2 Pos*
	0800-0331-9	0.705	1.025	0.560	0.785	0.524	23.171		16.836	HIV-2 Pos*
	0800-0331-10	0.356	0.567	0.356	0.495	0.473	13.193		16.124	HIV-2 Pos
	0800-0331-11	0.371	0.916	0.429	0.451	0.451	0.771		17.956	HIV-2 Pos
	0800-0331-12	0.458	0.502	0.349	0.604	0.516	0.524		15.244	HIV-2 Pos
	0800-0331-13	0.407	0.582	0.335	0.996	0.604	2.538		15.433	HIV-2 Pos
	0800-0331-14	0.429	0.553	0.371	1.040	0.640	0.887		17.098	HIV-2 Pos
	0800-0331-15	0.436	0.487	0.371	0.604	0.531	0.400		0.509	Neg

HIV-2 Pos* = HIV-2 Positive with reactivity to HIV-1 antigens

HIV-1 Group M Clades

Nine (9) major HIV-1 Group M clades and 13 CRFs consisting of a total of 96 samples as shown in **Table 6** from commercial sources were tested in singlicate with the HIV Profile™ Supplemental Assay. All 96 Group M clade samples tested positive for HIV-1 with 100% reactivity rate and a 95% confidence interval of 96.2% to 100%. Of the 96 samples tested, all 96 were tested as HIV-1 positive; one of the samples, a Clade C sample, was HIV-1 positive with reactivity to HIV-2 because of a weak reactive result for HIV-2 gp36.

Table 6: HIV-1 Group M Clade Samples

HIV-1 Group M Clade	Number of Samples	HIV Profile™ Supplemental Assay		
		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
CRF_01_AE	4	0	0	4
CRF02_AG	2	0	0	2
CRF18_cpx	1	0	0	1
CRF11_cpx	1	0	0	1
CRF25_cpx	1	0	0	1
CRF06_cpx	2	0	0	2
CRF14_BG	2	0	0	2
TOTAL	**96**	**0**	**0**	**96**

*One of the Clade C samples tested HIV-1 positive with reactivity to HIV-2 antigen

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

A total of 94 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 94 repeatedly reactive samples, twenty-one (21) were confirmed negative by a HIV 1/2 Supplemental Assay, seventeen (17) were confirmed negative by a HIV-1/HIV-2 Rapid* Test, and fifty-six (56) were confirmed negative by an IFA (Immunofluorescence Assay). The test results with the HIV Profile™ Supplemental Assay are summarized in **Table 7**.

Of these 94 samples, ninety-two (92) samples were negative and two (2) samples were indeterminate with the HIV Profile™ Supplemental Assay. None of these samples were confirmed positive with the HIV Profile™ Supplemental Assay. Overall, there was a 97.9% (92/94) concordance for negativity confirmation and 2.1% (2/94) indeterminate rate with this group of samples.

Table 7: Test Results of Repeatedly Reactive and Confirmed Negative Samples

	Supplemental Assay Results	**Number**	**HIV Profile™ Supplemental Assay**		
			Negative	**Indeterminate**	**Positive**
Samples Repeatedly Reactive with a Diagnostic or Screening HIV Test	HIV-1/2 Supplemental Assay NEG	21	21	0	0
	HIV-1/HIV-2 Rapid* Test NEG	17	17	0	0
	IFA NEG	56	54	2	0
	Total	**94**	**92 (97.9%)**	**2 (2.1%)**	**0**

*Rapid test approved for differentiation of antibodies to HIV-1 and HIV-2 as part of CDC HIV testing algorithm.

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

A total of 99 samples which were repeatedly reactive (RR) with an FDA approved or licensed HIV 1/2 assay but indeterminate with an FDA approved or licensed supplemental / confirmatory assay were tested with the HIV Profile™ Supplemental Assay. Of these 99 repeat reactive confirmed indeterminate samples, one was indeterminate with an FDA approved HIV 1/2 Supplemental assay and 98 were indeterminate with an FDA approved IFA (See **Table 8**).

The one sample confirmed indeterminate with the HIV 1/2 Supplemental assay was negative by the HIV Profile™ Supplemental Assay. Of the 98 samples confirmed indeterminate by IFA, 97 were negative and one, was indeterminate the HIV Profile™ Supplemental Assay.

In addition, fifteen (15) 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 HIV Profile™ Supplemental Assay. All of these 15 samples were confirmed to be positive by the HIV Profile™ Supplemental Assay.

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

	Supplemental Assay Result	Number	HIV Profile™ Supplemental Assay		
			Negative	Indeterminate	Positive
Samples Repeatedly Reactive with a Diagnostic or Screening HIV Test	HIV-1/2 Supplemental Assay IND	1	1	0	0
	IFA IND	98	97	1	0
	Total	**99**	**98 (99.0%)**	**1 (1.0%)**	**0 (0.0%)**
	IFA POS, Western Blot IND	15	0	0	15

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 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 HIV Profile™ Supplemental Assay (**Table 9)**.

Table 9: Test Results of Unspiked Samples with Potentially Interfering Factors

Potentially Interfering Factor	Number Tested	HIV Profile™ Supplemental Assay		
		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	10	0	0
TOTAL	**130**	**125 (96.15%)**	**4 (3.08%)**	**1 (0.77%)**

[a]HIV-2 positive, which was negative upon repeat testing in duplicate.

[b]HIV-1 indeterminate, which was negative upon repeat testing only 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 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 10**.

Table 10: Test Results of Potentially Interfering Factors Spiked HIV-1 Antibody

Potential Interfering Factor	Number Tested	HIV Profile™ Supplemental Assay			
		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 HIV Profile™ Supplemental Assay. Five (5) of these specimens were found to be positive for HIV-1 prior to spiking them with HIV-2 positive antibody. 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 11**).

Table 11: Test Results of Potentially Interfering Factors Spiked with HIV-2 Antibody

Potential Interfering Factor	Number Tested	HIV Profile™ Supplemental Assay			
		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 reactive for both HIV-1 and HIV-2 with the HIV Profile™ Supplemental Assay (**Table 12)**.

Table 12: Results of Potentially Interfering Factors Spiked with HIV-1 and HIV-2 Antibody

Potential Interfering Factor	Number Tested	HIV Profile™ Supplemental Assay		
		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 will have an adverse effect on the test results of the HIV Profile™ Supplemental Assay, an HIV negative human serum sample was spiked with 3600 ng/mL of extraneous biotin, which is three times the therapeutic level[8]. The HIV negative sample with or without 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 HIV Profile™ Supplemental Assay S/CO results in **Table 13**, the human serum sample containing 3600 ng/mL of biotin performed similarly to the same sample without added biotin, regardless of 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 test results.

Table 13: Test Results 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 HIV Profile™ Supplemental Assay. All 47 samples were negative, indicating that samples from these disease states did not result in cross reactivity in the HIV Profile™ Supplemental Assay (**Table 14**).

Table 14: Test Results on Additional Potentially Cross Reactive Samples

Disease State Samples	Number Tested	HIV Profile™ Supplemental Assay		
		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 HIV Profile™ Supplemental Assay. The test results showed that these substances did not cause cross reactivity in the HIV Profile™ Supplemental Assay (**Table 15**).

Table 15: Test Results on Additional Potentially Cross Reactive Samples

Panel Member Samples		Number Tested	HIV Profile™ Supplemental Assay		
			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 (96.30%)**	**1 (3.70%)**	**0 (0.00%)**

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

REPRODUCIBILITY

A reproducibility test panel consisting of five (5) samples with various antibody titers and the two kit controls were used for this study. The five member panel included 1 negative sample (R1) and four separate antibody positive samples specific for HIV-1 p65/p24 (R2), HIV-1 gp160 (R3), HIV-1 gp41 (R4), and HIV-2 gp36 or G5 (R5) all with antibody target levels contrived in normal human serum to be borderline with respect to the assay cutoff.

Each sample was tested in duplicate on one plate with each of 3 validation lots of kits and two runs per day for five days at Avioq and two external sites by one technician at each site. Thus, each sample was tested in a total of 180 replicates with 1 positive and 1 negative control per plate. The overall mean, SD and CV for each sample / antigen combination was calculated. Component variance analysis was conducted for each antibody sample/antigen combination to extract the variability attributable to kit lot, site, day and run. The results are shown in **Table 16**. Borderline results shown in highlighted bolded text for samples R2 – R5 are the target specific antigens to which each antibody sample was diluted.

Table 16: Test Results from the Reproducibility Study

				Within Run		Between Run		Between Days		Lot-to-Lot		Site-to-Site		Total Reproducibility	
Sample	Ag Well	Mean	n	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
PC	No Viral Ag	0.47	91	0.06	12.07	0.11	23.26	0.05	9.80	0*	0*	0.08	16.17	0.15	32.32
	p65	15.41	91	1.59	10.33	0*	0*	0.58	3.76	2.30	14.89	0.84	5.47	2.97	19.30
	gp160-D	1.19	91	0.12	10.06	0.06	4.69	0.11	9.36	0.07	5.95	0.07	5.70	0.20	16.69
	gp160	21.78	91	1.74	7.98	0*	0*	0.98	4.51	0.86	3.95	1.19	5.48	2.48	11.39
	gp41	16.18	91	2.20	13.60	0*	0*	0.67	4.13	1.75	10.84	0.65	4.04	2.96	18.33
	p24	14.65	91	1.80	12.31	0*	0*	0.74	5.06	2.04	13.92	1.38	9.40	3.14	21.43
	G5	8.92	91	0.89	10.01	0*	0*	0.42	4.71	0.77	8.68	0.80	8.95	1.49	16.67
NC	No Viral Ag	0.38	88	0.04	9.94	0.05	12.69	0.01	3.52	0*	0*	0.01	3.30	0.06	16.83
	p65	0.48	91	0.02	3.23	0.03	7.29	0*	0*	0.05	10.22	0.01	3.14	0.06	13.34
	gp160-D	0.33	91	0.05	14.67	0*	0*	0*	0*	0.01	4.06	0.00	1.13	0.05	15.26
	gp160	0.45	91	0.04	9.72	0*	0*	0*	0*	0.02	4.20	0.01	3.33	0.05	11.10
	gp41	0.47	91	0.04	9.43	0*	0*	0*	0*	0.01	3.08	0.00	0.84	0.05	9.95
	p24	0.37	91	0.03	6.87	0.05	12.26	0*	0*	0*	0*	0*	0*	0.05	14.06
	G5	0.37	91	0.04	11.76	0*	0*	0.02	5.52	0.01	2.38	0.01	3.49	0.05	13.66
R-1 (-)	No Viral Ag	0.37	180	0.05	12.26	0.04	10.20	0.01	3.31	0.02	4.33	0.01	3.72	0.06	17.26
	p65	0.42	180	0.06	14.85	0.04	8.70	0.03	6.22	0.02	5.44	0.02	4.17	0.08	19.54
	gp160-D	0.34	180	0.11	31.29	0.04	11.26	0.02	5.72	0*	0*	0.02	4.68	0.12	34.06
	gp160	0.41	180	0.08	18.65	0.04	8.67	0.04	9.64	0*	0*	0.03	7.71	0.10	23.99
	gp41	0.45	180	0.11	23.99	0.01	2.01	0.05	11.35	0.02	5.15	0.03	6.54	0.13	27.89
	p24	0.37	180	0.06	15.26	0.02	4.88	0.03	8.48	0*	0*	0.02	4.70	0.07	18.73
	G5	0.37	180	0.05	12.57	0.04	11.00	0.03	7.88	0*	0*	0.01	2.35	0.07	18.62
R-2 (+)	No Viral Ag	0.38	180	0.06	15.10	0.02	6.13	0.01	2.86	0*	0*	0.03	6.70	0.07	17.85
	p65	**1.35**	**180**	**0.08**	**5.71**	**0.08**	**5.73**	**0.07**	**5.22**	**0.13**	**9.28**	**0.07**	**5.34**	**0.19**	**14.40**
	gp160-D	0.37	180	0.04	10.21	0*	0*	0.02	6.16	0.02	4.63	0.01	2.15	0.05	12.97
	gp160	2.24	180	0.12	5.52	0.14	6.32	0.07	3.06	0.12	5.37	0.15	6.49	0.28	12.28
	gp41	0.90	180	0.06	6.83	0.04	5.00	0.03	3.30	0.05	5.14	0.04	4.61	0.10	11.41
	p24	**1.45**	**180**	**0.10**	**7.00**	**0.07**	**4.90**	**0.08**	**5.63**	**0.14**	**9.62**	**0.05**	**3.19**	**0.21**	**14.41**
	G5	0.36	180	0.06	15.33	0.03	7.61	0.03	7.21	0.01	2.50	0.02	5.42	0.07	19.51
R-3 (+)	No Viral Ag	0.38	180	0.05	13.51	0.04	10.78	0.01	2.72	0.01	2.53	0.02	6.42	0.07	18.81
	p65	0.98	180	0.07	6.84	0.07	6.64	0.05	5.34	0.05	5.49	0.04	4.48	0.13	13.03
	gp160-D	0.35	180	0.05	14.42	0.02	5.07	0.02	4.81	0*	0*	0*	0*	0.06	16.03
	gp160	**1.43**	**180**	**0.08**	**5.86**	**0.10**	**6.75**	**0.02**	**1.72**	**0.08**	**5.46**	**0.06**	**4.06**	**0.16**	**11.36**
	gp41	0.70	180	0.05	6.60	0.05	6.90	0.01	0.73	0.02	3.24	0.03	3.88	0.08	10.83
	p24	0.96	180	0.07	7.34	0.06	6.07	0.05	5.25	0.09	9.16	0.02	2.33	0.14	14.41
	G5	0.35	180	0.04	11.56	0.03	8.46	0.02	5.38	0.02	5.68	0.02	4.55	0.06	16.94
R-4 (+)	No Viral Ag	0.38	180	0.04	11.08	0.03	8.37	0.02	4.17	0.01	3.75	0.02	5.09	0.06	15.82
	p65	2.31	180	0.13	5.68	0.15	6.39	0.12	5.00	0.41	17.87	0.11	4.86	0.49	21.01
	gp160-D	0.38	180	0.03	9.14	0.02	5.03	0.02	4.97	0.01	3.12	0*	0*	0.05	11.97
	gp160	2.98	180	0.17	5.85	0.20	6.68	0.11	3.64	0.24	8.04	0.12	3.93	0.39	13.12
	gp41	**1.50**	**180**	**0.09**	**5.68**	**0.11**	**7.40**	**0.06**	**4.05**	**0.09**	**5.91**	**0.06**	**4.26**	**0.19**	**12.51**
	p24	0.83	180	0.06	7.27	0.05	5.90	0.04	5.14	0.10	11.75	0.04	4.28	0.14	16.45
	G5	0.36	180	0.06	15.73	0.02	4.25	0.04	11.38	0.02	5.12	0.02	6.16	0.08	21.42
R-5 (+)	No Viral Ag	0.38	180	0.04	9.41	0.04	9.59	0.03	7.28	0*	0*	0.02	4.21	0.06	15.85
	p65	0.47	180	0.04	8.80	0.03	6.79	0.03	6.77	0.04	7.84	0*	0*	0.07	15.19
	gp160-D	0.34	180	0.03	10.06	0.02	5.55	0.02	6.41	0*	0*	0.02	6.20	0.05	14.54
	gp160	0.43	180	0.05	12.18	0.00	0.92	0.02	5.34	0.01	1.88	0.03	6.22	0.06	14.83
	gp41	0.47	180	0.06	12.94	0.02	3.41	0.03	6.20	0.02	3.54	0*	0*	0.07	15.16
	p24	0.35	180	0.04	11.47	0.01	3.74	0.02	5.83	0*	0*	0.02	5.50	0.05	14.48
	G5	**1.24**	**180**	**0.08**	**6.49**	**0.07**	**5.42**	**0.06**	**5.20**	**0.12**	**9.99**	**0.12**	**10.05**	**0.22**	**17.30**

0* Negative variance component estimates were set to 0 and did not contribute to the total variance

CLINICAL SPECIFICITY / SENSITIVITY

CLINICAL SPECIFICITY

A total of 599 serum and plasma, including twenty (20) serum pediatric samples (ages 2 – 18 years), were collected from individuals at low risk for HIV infection and tested by the HIV Profile™ Supplemental Assay. Results are shown in **Table 17.** The overall clinical specificity in the low risk population was 98.16% (95% CI: 96.74% - 99.08%).

Table 17: Specificity of HIV Profile™ Supplemental 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, one was HIV-1 positive and another 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 502 samples collected from known HIV positive/AIDS patients, including 27 pediatric samples (ages 6 – 20 years), were tested with the HIV Profile™ Supplemental Assay. Results are shown in **Table 18.** One sample from an HIV-1 positive patient was negative by initial and repeat testing. This same sample was subsequently tested with an FDA approved HIV 1/2 Supplemental Assay and found to be HIV-2 positive with HIV-1 cross-reactivity. The overall clinical sensitivity was 99.8% (95% CI: 98.9% – 100%).

An earlier version of the device was used to conduct testing of 744 clinical samples (400 HIV-1 Positive, 202 HIV-2 positive, 10 HIV-1/HIV-2 coinfection, 15 HIV-1 Group O, 50 AIDs, 40 HIV-1 pediatric, and 27 HIV-1 Pregnant women) at five clinical sites, four external and one internal. Following updates to the device, a bridging study demonstrated that further testing for clinical sensitivity could be performed at a single internal site. The results of the internal testing are presented below for establishing clinical sensitivity.

Table 18: Sensitivity of HIV Profile™ Supplemental Assay in Known HIV Positive / AIDS Patients

Sample Type	Number	Positive	Indeterminate	Negative
HIV-1 Positive	266	265	0	1
HIV-1 Clades	21	21	0	0
HIV-2 Positive	125	125[a]	0	0
HIV-1/HIV-2 Coinfection	8	8[b]	0	0
HIV-1 Group O	10	10	0	0
AIDS	30	30	0	0
HIV-1 Positive Pediatrics	27	27	0	0
HIV-1 Positive Pregnant Females	15	15	0	0
TOTAL	**502**	**501 (99.8%)**	**0 (0%)**	**1 (0.2%)**

[a]Sixty-three (63) of 125 samples were interpreted as HIV-2 positive with reactivity to HIV-1 antigens. All other samples were HIV-2 positive.
[b]All samples were interpreted as HIV-2 positive with reactivity to HIV-1 antigens

ESTIMATION OF HIV INFECTION TIME

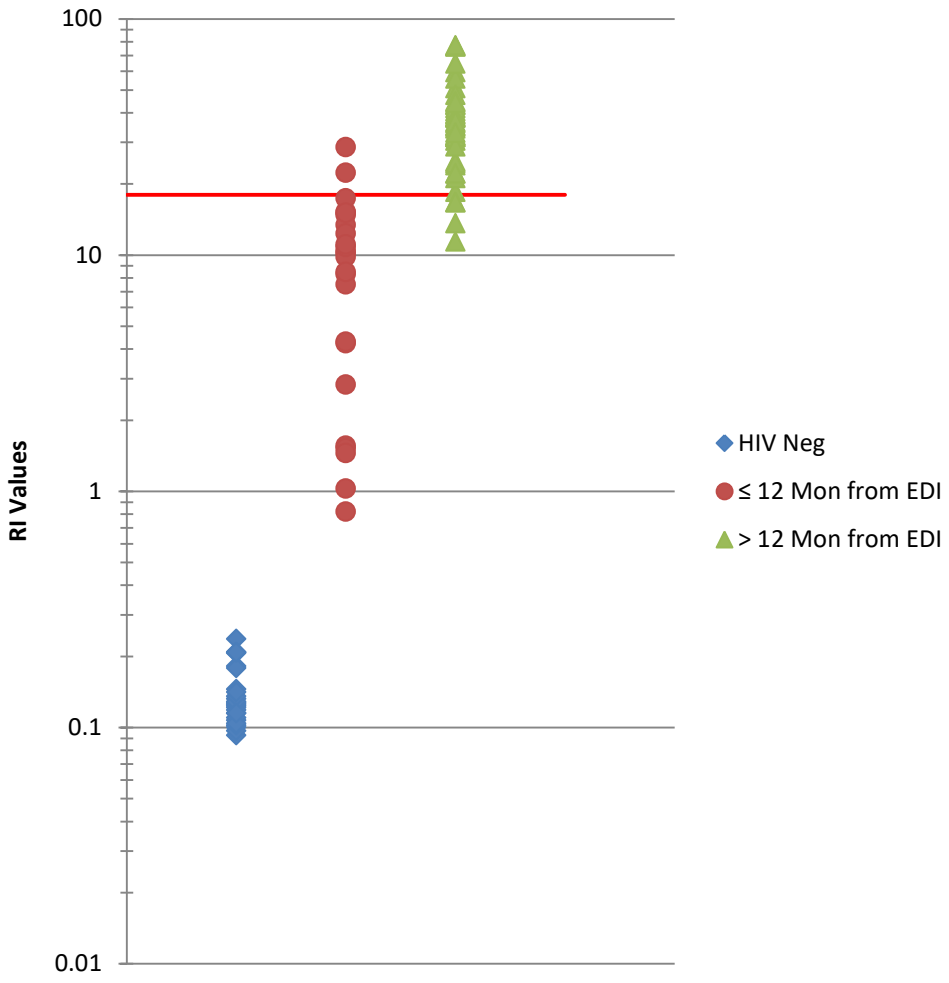
S/CO from two solid phase wells, one coated with normal (Well D) and the other coated with reduced concentrations (Well C) of HIV-1 gp160, was used to calculate a Recency Index (RI) for each sample. A Recency Index (RI) value is calculated as follows:

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.

REFERENCES

1. Hardy AM, Allen JR, Morgan WM, *et al.* The Incidence Rate of Acquired Immunodeficiency Syndrome in Selected Populations. *JAMA* 1985; 253(2):215-20.
2. Gallo RC, Salahuddin SZ, Popovic M, *et al.* Frequent Detection and Isolation of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and at Risk for AIDS. *Science* 1984; 224:500-3.
3. Brun-Vezinet F, Katlama C, Roulot D, *et al.* Lymphadenopathy associated virus type 2 in AIDS and AIDS-related complex. *Lancet* 1987; 1:128-32.
4. Quinn TC, Zacarias FRK, St. John RK, *et al.* AIDS in the Americas: an emerging public health crisis. *New Engl. J. Med.* 1989; 320:1005-7.
5. Markham P, Salahuddin SZ, *et al.,* Advances in the isolation of HTLV-III from patients with AIDS and AIDS-related complex and from donors at risk. *Cancer Res. (Suppl.)* 1985; 45:4588-91.
6. The U.S. Pharmacopeia 24/National Formulary 19: 1999; *Purified Water* : pg. 1753.
7. National Committee for Clinical Laboratory Standards: *Preparation and Testing of Reagent Water in the Clinical Laboratory*, Villanova, PA, NCCLS, 1997: C3-A3.
8. FDA Public Health Safety Communication November 28, 2017: *Biotin (Vitamin B7): Safety Communication-May Interfere with Lab Tests*

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

Avioq, Inc.
104 TW Alexander Drive
PO Box 12808
Research Triangle Park, North Carolina 27709
www.Avioq.com

EC REP

EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands

CE **2797**

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