

# **HUMAN IMMUNODEFICIENCY VIRUS TYPE 1**

## **Fluorognost™ HIV-1 IFA**

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A Qualitative Immunofluorescence Assay for the Detection of Antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) in Human Serum or Plasma Specimens, or Home Access HIV-1 Test System Dried Blood Spot Specimens.

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For in vitro diagnostic use

### **PRODUCT INSERT**

Catalog no. FG-25 25-test kit

Catalog no. FG-50 50-test kit

### **Manufactured and Distributed by**

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## NAME AND INTENDED USE

The Fluorognost HIV-1 Indirect Immunofluorescence Assay (IFA) is an *in vitro*, qualitative assay for the detection of antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) in human serum or plasma. It is intended to be used as an additional, more specific test for antibodies to HIV-1 in human serum or plasma specimens found to be repeatedly reactive by screening procedures, such as the Enzyme-Linked Immunosorbent Assay (EIA). Properly trained personnel can also use Fluorognost HIV-1 IFA as a screening test in hospital laboratories, medical clinics, physicians' offices and emergency care situations and in blood banks or other settings where enzyme immunoassays are not practical or available. Fluorognost HIV-1 IFA is also intended to be used as an additional, more specific test for antibodies to HIV-1 with dried blood spot (DBS) specimens obtained using the Home Access HIV-1 Test System and tested at authorized Home Access laboratories.

## SUMMARY AND EXPLANATION OF THE TEST

Human Immunodeficiency Virus Type 1 (HIV-1) is the major etiologic agent of Acquired Immunodeficiency Syndrome (AIDS). Current data indicate that the Acquired Immunodeficiency Syndrome (AIDS) is transmitted through sexual contact, through exposure to blood (including sharing contaminated needles and syringes) or through certain blood products, or when it is transmitted from an infected mother to her fetus or child during the perinatal period. Human Immunodeficiency Virus Type 1 (HIV-1) has been isolated from patients with AIDS and AIDS - related complex (ARC) and from healthy persons at high risk for AIDS. The prevalence of antibodies specific for HIV-1 infection in people not considered to be at increased risk is not known. Although there are two types of AIDS - causing viruses, namely, HIV-1 and HIV-2, infections with HIV-2 outside of West Africa are uncommon. The Fluorognost HIV-1 IFA is designed to identify the presence of HIV-1 virus specific antibodies.

The assay uses immortalized human T-cells which express HIV-1 antigens on their surface. The cells are fixed to the surface of an IFA glass slide. Fixed, uninfected T-cells are provided as a control. When a serum or plasma sample with HIV-1 antibodies comes in contact with the HIV-1 antigens on the slide, antigen/antibody binding takes place. Bound HIV-1 specific antibodies are detected with anti-human immunoglobulin conjugated to fluorescein isothiocyanate, which binds human antibodies and fluoresces when exposed to UV light. The interpretation of the degree and pattern of fluorescence of the infected cells of the IFA slide compared to the uninfected cells determines the HIV-1 status of the sample. Persons demonstrating antibodies to HIV-1 should be referred for medical evaluation, which may include testing by other techniques. A clinical diagnosis of AIDS can be made only if a person meets the case definition of AIDS established by the Centers for Disease Control.

Some individuals infected with HIV-2 may have antibodies that will recognize and bind to viral antigens present on the Fluorognost HIV-1 IFA slides. However, the degree and pattern of fluorescence may vary, even after seroconversion. **The Fluorognost HIV-1 IFA is not intended to identify HIV-2 antibodies or to distinguish HIV-2 from HIV-1.**

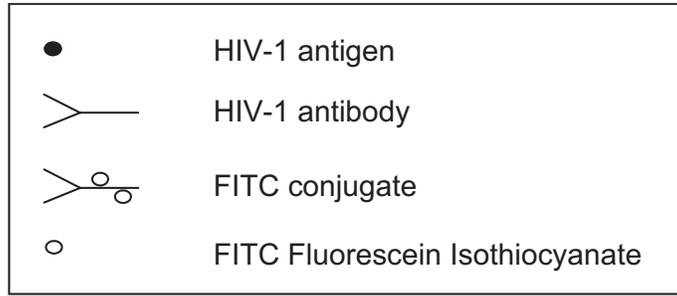
## CHEMICAL AND BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The Fluorognost HIV-1 IFA is a test for HIV-1 antibodies based on specific antibody binding to HIV-1 (HTLV-III B isolate) antigens expressed on the surface of immortalized human T-cells fixed to a glass slide. T-cells not expressing HIV-1 antigens serve as controls for each test. Slides are processed to inactivate any virus associated with infected cells, including complete air drying, refrigerated acetone treatment to remove the lipid components of the virus, and dehydration through evaporation of the acetone solvent. This inactivation process fixes both the HIV-1 infected and uninfected cells to their respective cell wells on the glass IFA slide.

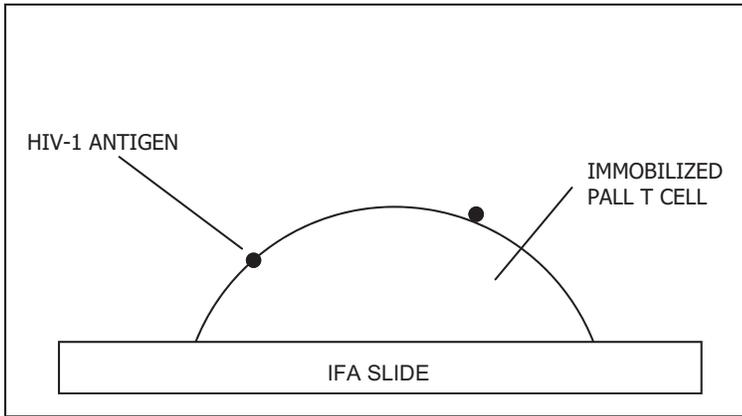
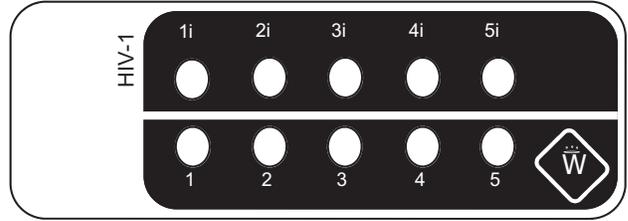
To perform the assay, a serum or plasma specimen or Home Access HIV-1 Test System DBS specimen is diluted and placed in the infected and uninfected cell well of the IFA slide and incubated. If HIV-1 antibodies are present in the sample, they will bind to HIV-1 viral antigens present on the infected cell surface. Unbound material is removed by aspiration and washing. Antibody to human immunoglobulin conjugated with fluorescein isothiocyanate (FITC) is added to the HIV-1 infected and uninfected cell wells of the IFA slide, and again incubated. Unbound material is removed by aspiration and washing. A glass cover slip is mounted to the IFA slide and the slide is viewed under a microscope with UV light.

If antibodies to HIV-1 are present in the serum or, plasma specimen or Home Access HIV-1 Test System DBS specimen, a characteristic pattern of fluorescence will become visible under ultraviolet light. Comparing and differentiating the pattern and intensity of fluorescence in the uninfected and infected cell well for each sample allows the interpretation of this fluorescence.

**EXHIBIT 1: DIAGRAM OF THE FLUOROGNOST HIV-1 IFA ASSAY PROCEDURE**

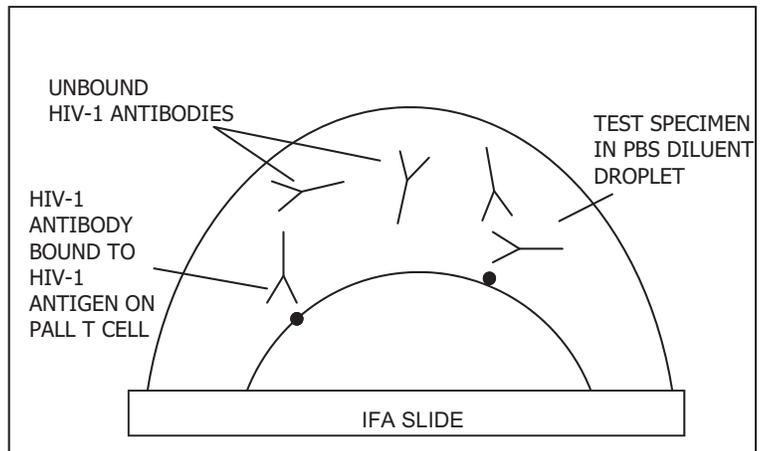


**Figure 1**  
The Fluorognost HIV-1 IFA slide contains two adjacent rows of cell wells. The red Teflon-coated row, labeled 1i-5i, carries infected HIV-1 cells. The blue-coated row, labeled 1-5, carries uninfected control cells. The frosted end of the slide to the left of the red HIV label is provided for handling the slide.



**Figure 2**  
The human Pall T Cells expressing HIV-1 antigens are firmly attached to positions 1i-5i on the Fluorognost HIV-1 slide. The IFA slides have been permeabilized and virally inactivated by solvent treatment.

**Figure 3**  
A 15µl droplet of diluted test specimen is added to both the HIV-1 infected and uninfected cell wells. If antibodies to HIV-1 are present in the blood sample, specific HIV-1 antigen / antibody binding will occur in the HIV-1 infected cell well. No binding will occur in the uninfected control well. Unbound HIV-1 antibodies are removed during the washing step.



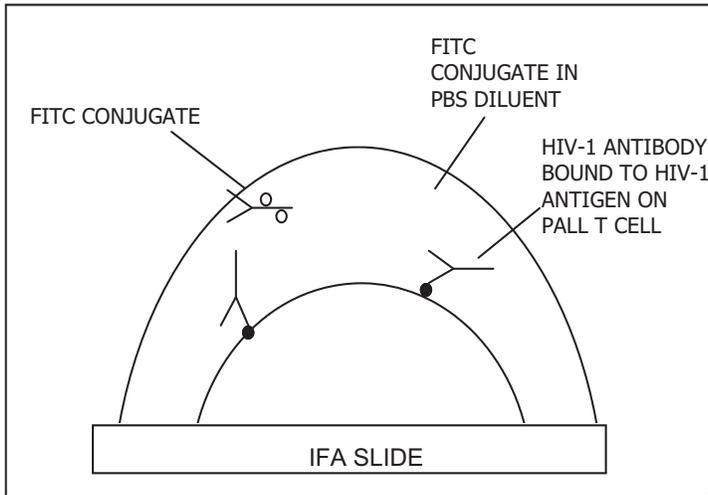


Figure 4  
Following the washing step, a 15µl droplet of the FITC conjugate working dilution is added to both the HIV-1 infected and uninfected cell wells and incubated. The conjugate, which is anti-human IgG (rabbit) antibody, is covalently bound to the fluorochrome, FITC (fluorescein isothiocyanate).

Figure 5  
If HIV-1 antibodies are present in the test specimen, they bind to the HIV-1 antigen on the PALL T cell, and are in turn bound by the antihuman FITC conjugate, forming a multiple sandwich structure. Unbound FITC conjugate is removed by the second washing step.

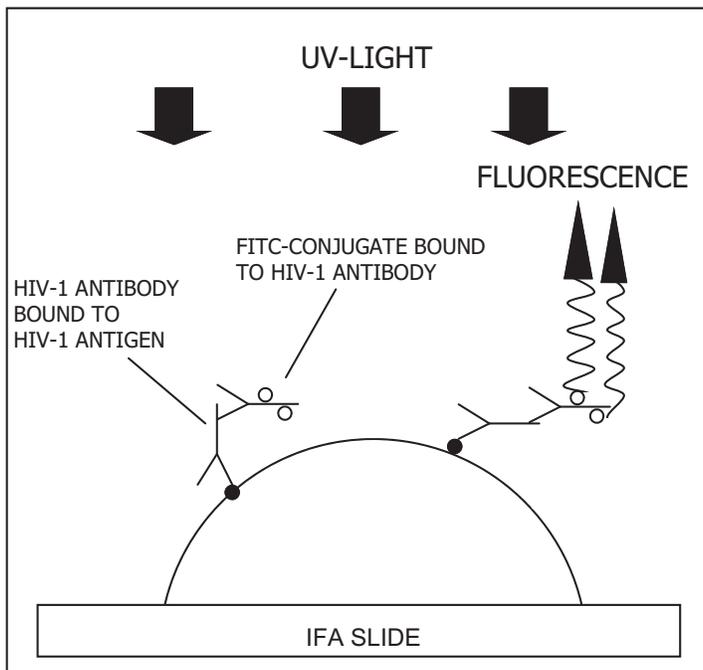
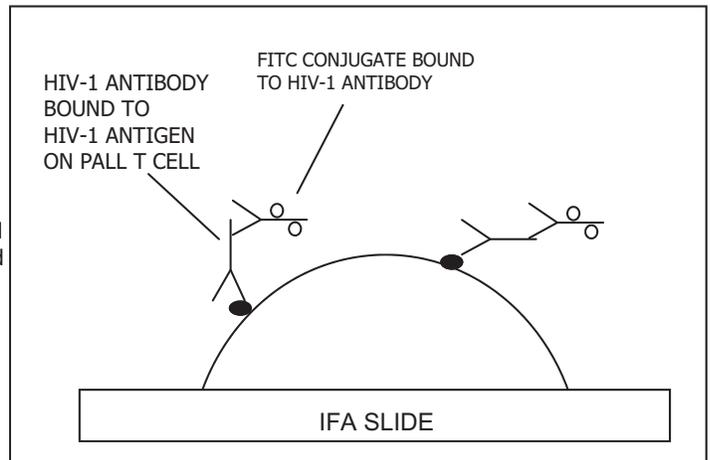


Figure 6  
The binding of FITC conjugate in the HIV-1 infected cell well demonstrates the presence of HIV-1 antibodies in the test specimen. The presence of the FITC conjugate / HIV-1 antibody / HIV-1 antigen sandwich structure is detected by the fluorescence that is emitted by the fluorochrome upon excitation with ultraviolet light. The resulting fluorescence allows the cellular HIV-1 antigen expression patterns to be visible. These patterns remain obscure if the test specimen does not contain HIV-1 antibodies.

## REAGENT DESCRIPTION (materials provided)

The Fluorognost HIV-1 IFA is available as a 25 or, 50 Test Kit

Component	50-Test Kit	25-Test Kit
1. <b>IFA Slides</b> have inactive HIV-1 infected and uninfected control cell wells capable of detecting the presence or absence of HIV-1 antibodies	10 IFA slides, each containing parallel rows of 5 HIV-1 infected wells numbered (1i-5i) and 5 uninfected wells (1-5)	5 IFA slides, each containing parallel rows of 5 HIV-1 infected wells numbered (1i-5i) and 5 uninfected wells (1-5)
2. <b>Concentrated Diluent</b> consisting of 30 ml of liquid phosphate buffered sodium chloride in a screwed plastic vial to make 1 liter of phosphate buffered saline (PBS) pH 7.3.	Two screwed plastic vials containing 30ml of liquid phosphate buffered saline each for 1 liter PBS.	Two screwed plastic vials containing 30ml of liquid phosphate buffered saline each for 1 liter PBS.
3. <b>Concentrated FITC-Conjugate</b> consisting of 300 µl of a concentrated solution of fluorescein isothiocyanate conjugated with a Rabbit Anti-Human IgG, in a plastic vial with green color-coding. To be diluted with PBS for a working dilution.	One plastic vial containing 300 µl of Concentrated FITC Conjugate with green color-coding on the cap.	One plastic vial containing 300 µl of Concentrated FITC Conjugate with green color-coding on the cap.
4. <b>Mounting Media</b> consisting of a 3.0 ml solution of ready to use PBS buffered glycerol in a plastic dispenser bottle with cap.	One plastic dispenser bottle containing 3.0 ml of PBS buffered glycerol.	One plastic dispenser bottle containing 3.0 ml of PBS buffered glycerol.
5. <b>Negative Serum Control</b> consisting of 20 µl of heat inactivated, human serum, negative for antibody to HIV-1. Non-reactive for Hepatitis B surface antigen and antibody to Hepatitis C virus. Preserved with 0.1% sodium azide in a plastic screw cap vial. To be diluted with PBS Diluent.	One plastic vial containing 20 µl of Negative Serum Control.	One plastic vial containing 20 µl of Negative Serum Control.
6. <b>Positive Serum Control</b> consisting of 20 µl of heat inactivated, human serum, positive for antibody to HIV-1. Non-reactive for Hepatitis B surface antigen and antibody to Hepatitis C virus. Preserved with 0.1% sodium azide in a plastic vial with a <b>RED Screw Cap</b> . To be diluted with PBS Diluent.	One plastic vial with a <b>RED CAP</b> containing 20 µl of Positive Serum Control.	One plastic vial with a <b>RED CAP</b> containing 20 µl of Positive Serum Control.
7. <b>Glass Cover slip</b> package consisting of 5 Cover slips. One Cover slip is used for each IFA slide.	Two packages of Cover slips, each containing 5 Cover slips.	One package of Cover slips, containing 5 Cover slips.

**WARNINGS FOR USERS**  
**For In Vitro Diagnostic Use**

**WARNING: FDA has licensed this test kit for use with serum or plasma specimens, or Home Access HIV-1 Test System DBS specimens only. Use of this licensed test kit with specimens other than those specifically approved for use with this test kit may result in inaccurate test results.**

**HANDLE PATIENT SPECIMENS, IFA SLIDES AND FLUOROGNOST IFA SERUM CONTROLS AS IF CAPABLE OF TRANSMITTING AN INFECTIOUS AGENT.**

HIV-1 infected T-cells have been fixed onto the infected cell wells of the IFA slide and virally inactivated by acetone and dehydration. The **NEGATIVE AND POSITIVE SERUM CONTROLS** have been virally inactivated by heat treatment. In addition, serum used to produce the **CONTROLS** was shown to be non-reactive for Hepatitis B surface antigen and antibodies to Hepatitis C virus. However, no known test method can offer assurance that products derived from human blood will not transmit infectious agents. **Therefore these components must be handled as if they are capable of transmitting infectious agents.**

Do not pipet by mouth. Avoid splashing or forming aerosols with specimens.

Wear disposable gloves throughout the test procedure. Dispose of gloves as biohazardous waste. Thoroughly wash hands after handling test reagents.

Do not smoke, eat or drink in areas where specimens or kit reagents are handled.

Wipe spills promptly with a 1:4 dilution of liquid household bleach and allow standing at least 60 seconds for disinfection.

Dispose of all specimens and material used in the Fluorognost HIV-1 IFA procedure as biohazardous waste. The recommended method of solid waste disposal is autoclaving for a minimum of 1 hour at 121°C. Disposables may be incinerated. Contaminated liquid materials should be mixed with a 1:4 dilution of household bleach. Allow the material to stand for at least 60 minutes for disinfection prior to disposal. **WARNING: DO NOT AUTOCLAVE LIQUIDS CONTAINING BLEACH.**

Clamp all hoses firmly to the vacuum pump and traps to prevent separation during aspiration.

## PRECAUTIONS FOR USERS

1. **DO NOT INTERCHANGE REAGENTS BETWEEN KIT LOTS.**
2. **Do not use a kit beyond its expiration date.** The date is printed on kit boxes.
3. Bring all kit reagents and specimens to room temperature (20-30°C) before use (approximately 30 minutes).

Keep all open reagents refrigerated (2-8°C) when not in use. Do not store slides after their pouch has been opened.

Use aseptic technique when opening and withdrawing aliquots from the reagent vials. Avoid microbial contamination and cross-contamination of reagents. **Do not use reagents which are cloudy and reveal microbial growth.**

Every slide is packaged along with a desiccant. Properly discard desiccant upon slide removal.

Avoid the use of test specimens which are contaminated by microbial growth, or which have been frozen and thawed more than twice. See "Specimen Collection and Storage" section for details.

Use only the serum controls supplied with the kit.

It is recommended that a separate pipette tip be used for each specimen and each reagent to avoid cross-contamination. In particular, trace contamination of the Negative Control, or any specimen not containing antibodies to HIV-1, will give rise to erroneous false positive results when compared with the Positive Control. Contamination of the FITC-Conjugate by any human serum will cause partial or complete neutralization of the Conjugate and may give rise to erroneous and negative results.

Do not interchange vial or bottle caps or stoppers; this will lead to cross-contamination of reagents. For ease of use, the Positive Serum Control is color coded with red labeling and has a **RED** screw cap. The Negative Serum Control has a **CLEAR** screw cap. Always designate separate reservoirs for specific reagents and specimens and/or their dilutions.

Handle the Fluorognost HIV-1 IFA slides by the rim or by the frosted end of the slide only. Never touch the IFA slide cell wells where HIV-1 infected and uninfected cells have been attached. Handle the sealed individual slide pouches without applying pressure to the labeled side of the slide.

Use distilled water to dilute reagents in order to avoid substances that may interfere with the assay.

Adherence to the procedures outlined within the Product Insert in the kit or in the Instructions for Laboratory Training and Qualification Brochure is necessary for the successful use of this product. When properly conducted, the Fluorognost HIV-1 IFA will accurately demonstrate the presence or absence of HIV-1 antibodies. While this is a criterion for infection by HIV-1, it must not be considered equivalent to a diagnosis of Acquired Immunodeficiency Syndrome (AIDS). Such a diagnosis can only be made by a physician according to accepted medical criteria.

Wash hands thoroughly after handling test reagents and on completion of the assays.

FDA has licensed this test kit for use with serum or plasma specimens or Home Access HIV-1 Test System DBS specimens only. Use of this licensed test kit with specimens other than those specifically approved for use with this test kit can result in an inaccurate test result.

UV light will cause FITC-Conjugate Fluorescence to fade with time. Be careful not to expose each microscopic field to UV light for over 15-30 seconds.

Make sure that the UV microscope, filters, objectives, and light sources are operating properly and that the microscope attachments are adjusted and aligned correctly.

### **STORAGE CONDITIONS**

Store all unopened kits and reagents at 2-8°C.

### **INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS**

Changes in the physical appearance of the reagents supplied may indicate instability or deterioration of these materials. Do not use any reagent that is cloudy and/or reveals microbial growth.

## SPECIMEN COLLECTION AND PREPARATION

Handle all specimens as if capable of transmitting infectious agents.

Fluorognost HIV-1 IFA is licensed for use with DBS specimens obtained using the Home Access HIV-1 Test System and tested at authorized Home Access testing laboratories using proprietary procedures. Serum specimens should be removed from the clot soon after collection to minimize hemolysis.

Plasma specimens may be collected by standard procedures. Laboratory studies have demonstrated that plasma prepared from blood collected with citrate, EDTA, ACD, CPD, or CPDA, as anticoagulant or specimens obtained from serum separator tubes, performs in the same way as does serum; however, an increased incidence of indeterminate results has been observed with heparinized plasma.

No clinically significant effect has been detected in performance of the assay in the presence of hemolysis (hemoglobin < 1.8 g/dl) or lipemia (triglycerides < 500 mg/dl). The presence of gross hemolysis or lipemia may interfere with the evaluation of the IFA slides, and those specimens should be recollected if possible. If any particulate matter is observed, it should be removed by a brief centrifugation before the assay.

Serum or plasma may be stored under refrigeration at 2-8°C for up to one week or may be stored frozen. Frozen specimens (frozen -20°C for up to 5 years) should be allowed to thaw completely, mixed well and centrifuged, if necessary, to remove any visible particulate matter. Allow the specimens to come to room temperature before beginning the assay. The test specimens should not be subjected to freezing and thawing more than twice. If specimens are shipped, they should be shipped in accordance with the requirements for transporting etiological agents.

## MATERIALS REQUIRED BUT NOT PROVIDED

1. Dilution tubes, 12 mm x 75 mm (3"), with screw cap or stopper is recommended.
2. Micropipettes with a volume range of 1 -20  $\mu$ l and 1 -200  $\mu$ l are recommended.
3. Staining dish / slide holder. This accessory is helpful for immersion of the IFA slides into the PBS Diluent and for separation between the slides.
4. A rotary or shaking platform is required for the washing steps.
5. An incubator capable of maintaining  $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$  is required.
6. Humidity chamber. A 6" to 7" petri dish with cover can be used with a wetted layer of cloth or filter paper serving as a reservoir. Allow the humidity chamber to equilibrate for 30 minutes at  $37^{\circ}\text{C}$ .
7. Distilled water is required for the preparation of the PBS Diluent.
8. A volumetric flask with a volume of at least 1.0 liter is required for the preparation and storage of the PBS diluent.
9. A fluorescence microscope with an epifluorescent attachment using an incident light fluorescence is required with the following hardware, or its equivalent.

Light source:	Mercury vapor lamp 50 - 200W
Excitation filter:	KP 500
Splitting mirror:	TK510
Barrier filter:	K510 or K530
Suppression filter:	BG – 38
10. A vacuum pump or other aspiration device is needed to remove liquid from the IFA slide wells. A chlorine bleach trap should be attached to the vacuum line.
11. A timer capable of measuring 10 minutes and 30 minutes is recommended.
12. A micro tube centrifuge is optional for removal of particulate matter from specimens.

## PRELIMINARY CRITICAL PROCEDURES

Construct a humidity chamber as described in the Section "Materials Required But Not Provided".

Turn on the mercury vapor lamp of the fluorescence microscope at least 15 minutes before the first observation. The UV microscope should be fitted with the hardware and accessories described in the section of the product insert titled "Materials Required But Not Provided".

Connect a vacuum pump and chlorine bleach trap or other aspiration device to remove liquid waste from the IFA slides.

Bring all reagents and the IFA slides to ambient temperature prior to testing.

## SERUM AND PLASMA ASSAY PROCEDURE

### ASSAY SET-UP (STEPS 1-5)

All procedural steps should be followed precisely as written. Failure to do so may result in aberrant test results.

Step 1: Bring all reagents to room temperature (20-30° C) before use (approximately 30 minutes).

Step 2: Record the Lot Number and Expiration Date of the Kit on the Fluorognost HIV-1 IFA Worksheet. Test specimen codes and results should also be recorded on the Worksheet.

Step 3: Construct a humidity chamber as described in the Section "Materials Required But Not Supplied".

Step 4: Turn on the mercury vapor lamp of the fluorescence microscope at least 15 minutes before the first observation. The UV microscope should be fitted with the hardware and accessories described in the Section of the Product Insert titled "Materials Required But Not Supplied".

Step 5: Connect a vacuum pump and chlorine bleach trap or other aspiration device to remove liquid waste from the IFA slides.

### PREPARATION OF REAGENTS (STEPS 6-8)

#### Step 6: PHOSPHATE BUFFERED SALINE DILUENT (PBS)

Completely dilute contents of the Concentrated PBS Diluent Container to 1.0 liter of distilled water. Mix well. CAUTION: The PBS Diluent does not contain a preservative. To prolong the integrity of the PBS Diluent, prepare PBS Diluent with sterile distilled water. After each use, the Diluent has to be sterile filtered into a new sterile container before storing at 2-8° C.

#### **NOTICE TO USERS**

Each individual who intends to report results of the Fluorognost HIV-1 IFA is strongly advised by the manufacturer to qualify as a reader by completing the program outlined in the Instructions for Laboratory Training and Qualification.

Discard if there are visible signs of microbial contamination. Do not reuse the reconstituted Diluent after 8 months.

### Step 7: FITC-CONJUGATE

- a. On day of use, dilute 15 µl of FITC-Conjugate (green screw cap) with 135 µl of PBS Diluent per slide in a sealable centrifuge tube. Gently mix by inverting the closed vial several times. DO NOT SHAKE.
- b. Avoid exposing the FITC-Conjugate to direct sunlight or extremely bright illumination.
- c. Store at 2-8° C for subsequent tests.

### Step 8: SERUM CONTROLS

- a. On day of use, dilute 5 µl of Positive Serum Control (red screw cap) with 145 µl of PBS Diluent per 5 slides to form a working dilution. Gently mix by inverting the closed vial several times.
- b. On day of use, dilute 5µl of Negative Serum Control with 145 µl of PBS Diluent per 5 slides to form a working dilution. Gently mix by inverting the closed vial several times.
- c. Store at 2-8°C.

## **DILUTION OF TEST SPECIMENS (STEP 9)**

### Step 9: TEST SPECIMEN WORKING DILUTION

Add 300 microliters of PBS Diluent to 10 microliters of test specimen in a tube with a conical bottom and cap to prepare working dilution. Use on the day of dilution.

## **ASSAY PROTOCOL (STEPS 10-25)**

Step 10: Remove the number of Fluorognost HIV-1 IFA slides required for the planned testing from the Kit container. Although each IFA slide can process five specimens, each run must be validated by the use of one Positive and one Negative Serum Control. Hence, the Kits of 25 and 50 Tests can process 23 and 48 test specimens, respectively. The required number of slides may be calculated as follows:

$$\text{Number of slides} = \frac{\text{Number of specimens} + 2}{5}$$

Both the IFA slide and Cover slip sachets have been designed to allow opening without the use of scissors. Simply pull the flaps of the sachet apart gently and remove the IFA slide, touching only the frosted end and/or rim of the slide.

**CAUTION: Remember to identify each IFA slide by current date and specimen codes before applying test specimen dilutions. The frosted end of the slide may be written on with a pencil or a waterproof laboratory marker pen.**

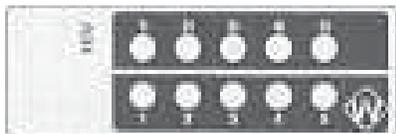


Exhibit 2: Fluorognost HIV-1 IFA slide

Step 11: Each IFA slide contains two parallel rows of cell wells. The HIV-1 infected cell wells (1i-5i) are surrounded by a RED Teflon coating and the uninfected control cell wells (1 -5) are surrounded by a BLUE Teflon coating. A Mute test specimen is placed in the corresponding HIV-1 infected and uninfected cell wells.

Step 12: Place 15 microliters of the diluted Positive Serum Control in the cell well labeled "1i" (infected cells in RED colored upper row of the IFA slide). Place 15 microliters in the cell well labeled "1" (uninfected cells in BLUE colored lower row of the IFA slide).

Step 13: Place 15 microliters of the diluted Negative Serum Control in the cell well labeled "2i" (infected cells in RED colored upper row of the IFA slide). Place 15 microliters in the cell well labeled "2" (uninfected cells in BLUE colored lower row of the IFA slide). If Serum Controls are placed in a different arrangement be sure to place the Negative Serum Control between any test specimen and the Positive Control.

Step 14: Add 15 microliters of the first diluted test specimen to the cell well labeled "3i" (infected cells in RED colored upper row of the IFA slide). Add 15 microliters to the cell well labeled "3" (uninfected cell well in the BLUE colored lower row of the IFA slide). Additional test specimens should be placed in corresponding sequential slide wells e.g. 4i/4, 5i/5, etc.

**CAUTION: CAREFULLY EXAMINE THE SLIDE TO BE SURE THAT SAMPLE WAS ADDED TO EACH WELL. DO NOT CONTACT THE CELL LAYER WITH THE PIPET TIP AND DO NOT ALLOW THE CELL WELLS TO DRY.**

Step 15: Following specimen application, transfer the IFA slide to the humidity chamber and incubate at 37°C for 30 minutes (see Step 3, humidity chamber). **CAUTION: DO NOT TILT OR SHAKE THE SLIDE DURING TRANSFER TO AND FROM THE HUMIDITY CHAMBER OR INCUBATOR AS CROSS-CONTAMINATION CAN OCCUR WHICH MAY LEAD TO FALSE POSITIVE RESULTS.**

Step 16: Following incubation, carefully remove the slide from the humidity chamber and thoroughly aspirate each of the cell wells of the IFA slide on a level surface. Be careful not to scratch the cell well surfaces.

Step 17 First Wash - Briefly rinse the IFA slide in a beaker containing PBS Diluent and immediately place the IFA slide into the slide holder containing a fresh volume of PBS Diluent. Make sure that all cell wells on the IFA slide are submerged. Then place the slide holder on a rocking or rotary platform for 10 minutes at room temperature.

Step 18: Following the first washing step, remove the slide from the slide holder and carefully aspirate the liquid from each cell well and/or blot the surrounding area, making sure that the entire surface of the IFA slide is free from excess fluid.

Step 19: Place 15 microliters (0.015 ml) of the diluted FITC-Conjugate in each IFA slide well position containing a test specimen or Serum Control.

Step 20: Transfer the IFA slide to the humidity chamber and incubate at 37°C for 30 minutes. **NOTE: THERE IS NO FURTHER RISK OF CROSS-CONTAMINATION.**

Step 21: Following incubation, remove the slide from the humidity chamber and aspirate the liquid from each of the cell wells.

Step 22: Second Wash - Briefly rinse the IFA slide in a beaker containing PBS Diluent and immediately place the IFA slide into a slide holder containing fresh PBS Diluent. Make sure that all cell wells on the IFA slide are submerged. Then place the slide holder on a rocking or rotary platform for 10 minutes at room temperature. **CAUTION: DO NOT ALLOW THE IFA SLIDE TO DRY.**

Step 23: Following the second washing step, remove the slide from the slide holder and carefully aspirate all excess liquid off the IFA slide.

Step 24: Place one drop of Mounting Media in each well to insure that each cell spot is covered. Align and cover each IFA slide with a Cover slip (one Cover slip per slide). Gently press down on the Cover slip making sure there are no air bubbles. For correct microscopic evaluation of the IFA cell wells the Mounting Media must completely fill the space between each IFA slide cell well position and the Cover slip.

At this point, the IFA slide is ready for evaluation and interpretation of the assay results. It is strongly recommended that the IFA slide(s) be evaluated immediately after the mounting procedure is completed. **CAUTION: THE IFA SLIDES MUST BE CAREFULLY PROTECTED FROM BRIGHT LIGHT TO AVOID A GRADUAL BLEACHING OF THE FLUORESCIN.**

## QUALITY CONTROL

The Fluorognost HIV-1 IFA Positive and Negative Serum Controls included in the Kit must be included with each run, regardless of the number of specimens tested. To validate the IFA run, first examine the Negative Serum Control, inspecting both the uninfected and HIV-1 infected cells in that order. Then examine the Positive Serum Control inspecting the uninfected and infected cells as above. The following is a description of the expected appearance of the Serum Controls.

**1. Negative Serum Control:** The contours of the individual cells in the Negative Control Serum in both the uninfected **BLUE** cell well "2" and the HIV-1 infected **RED** cell well "2i" should be barely visible and the cytoplasm of the cells must not exhibit any specific fluorescent staining greater than the surrounding cellular matrix. The appearance of the uninfected and infected cells of the Negative Control should be virtually indistinguishable. See exhibit 3 illustrating the appearance of the uninfected and infected cells of a typical Negative Serum Control.

**2. Positive Serum Control:** The uninfected cells located in the **BLUE** cell well "1" must not exhibit any specific cytoplasmic staining greater than the surrounding cellular matrix and should have an appearance similar to that seen with the Negative Serum Controls located in the **BLUE** cell well "2" and the **RED** cell well "2i". In contrast, the HIV-1 infected cells of the Positive Control located in **RED** cell well "1" should exhibit an intense apple green staining of the cytoplasm which can be easily differentiated from the uninfected cells.

Please note that as an additional internal control, the HIV-1 infected cell well is a "mixed cell environment" containing 40-70% infected PALL T-cells mixed with uninfected PALL T-cells. As a result, the uninfected cells within the infected cell well of the Positive Control will not exhibit specific fluorescent staining greater than the surrounding cellular matrix. See exhibit 4 illustrating the uninfected and infected cells of typical Positive Serum Control.

## INDICATIONS OF INSTABILITY OR DETERIORATION OF THE IFA SLIDE

The **Negative Serum Control** must not exhibit any specific fluorescent staining in either the HIV-1 infected or the uninfected cells. The **Positive Serum Control** must demonstrate a typical positive cytoplasmic fluorescence pattern in the HIV-1 infected cells. The uninfected cell well of the **Positive Serum Control** must **not** exhibit any specific fluorescent staining and should have an appearance similar to that seen with the **Negative Serum Control**. If any IFA result is not in accordance with the aforementioned criteria, deterioration of the Fluorognost HIV-1 IFA slides, the Positive or Negative Serum Control, or the FITC-Conjugate may be suspected. Any Kit showing deviation from the criteria, or showing an atypical fluorescence pattern with the Positive and/or Negative Serum Control should not be used.

## INTERPRETATION OF RESULTS

NOTE: VALIDATION OF THE NEGATIVE AND POSITIVE SERUM CONTROLS IS REQUIRED FOR THE IFA RESULTS TO BE CONSIDERED VALID.

The presence or absence of antibodies to HIV-1 in a test specimen is determined by the subjective comparison and differentiation of the intensity and the pattern of fluorescence between the uninfected control cells and the HIV-1 infected cells. The IFA Test result is then interpreted as **NEGATIVE, POSITIVE, OR INDETERMINATE**.

Evaluation and interpretation of a test result should be, carried out for each test specimen in the following sequence:

**First**, evaluate the **UNINFECTED CONTROL CELLS (BLUE)** of the test specimen for fluorescent staining. There should not be any specific cytoplasmic staining greater than the surrounding cellular matrix and the cells should be similar in appearance to the uninfected and infected cells of the Negative Serum Control (**BLUE** cell well "2" and **RED** cell well "2i") and the uninfected cells of the Positive Serum Control (**BLUE** cell well "1").

**Second**, evaluate the **HIV-1 INFECTED CELLS (RED)** of the test specimen for fluorescent staining. Use the following interpretation criteria to evaluate and interpret the IFA test result:

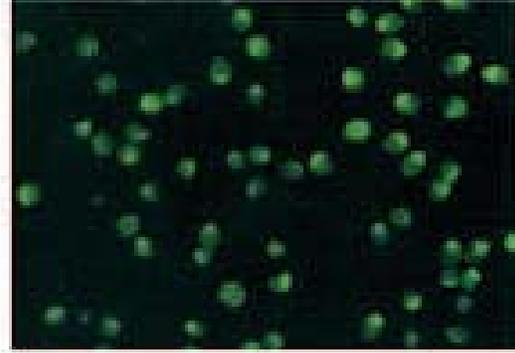
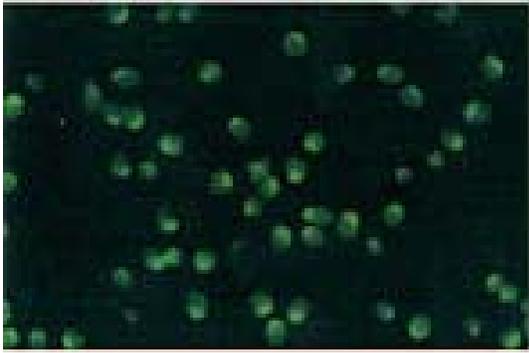
### NEGATIVE RESULT INTERPRETATION CRITERIA

A test specimen is interpreted as **NEGATIVE** when there is **no specific fluorescent staining** of the infected cells and there is no significant difference in the intensity of fluorescent staining and the pattern of fluorescence between the HIV-1 infected and uninfected cells. **The test specimen is reported as negative and no follow up testing is required.**

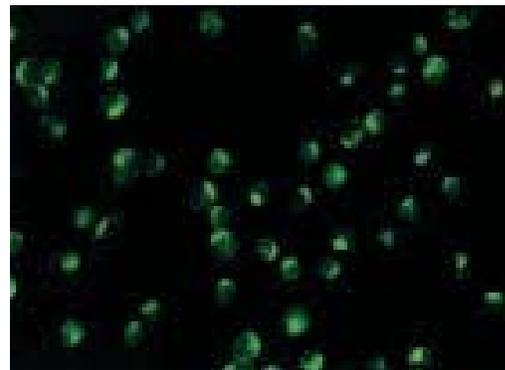
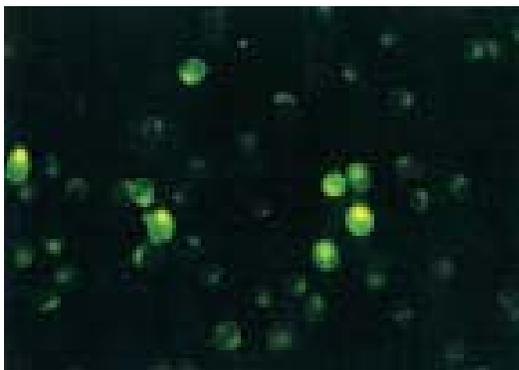
**DESCRIPTION OF NEGATIVE IFA RESULT:** In test specimens having a **NEGATIVE** IFA result interpretation, both the uninfected and infected cells of the test specimen will have an appearance similar to the uninfected and infected cells of the Negative Serum Control and the uninfected cells of the Positive Serum Control.

### POSITIVE RESULT INTERPRETATION CRITERIA

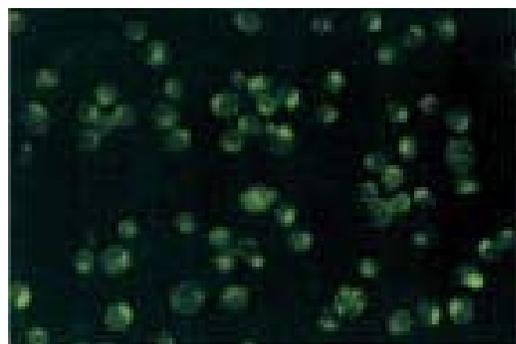
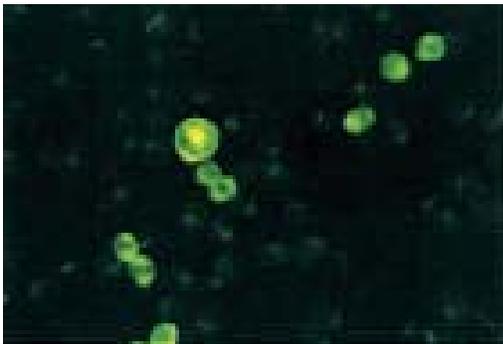
A test specimen is interpreted as **POSITIVE** when there is a **specific cytoplasmic staining pattern** in the HIV-1 infected cells and there is a significant difference in the intensity of fluorescent staining and the pattern of fluorescence between the HIV-1 infected and uninfected cells. **The test specimen is reported as positive and no follow up testing is required.**



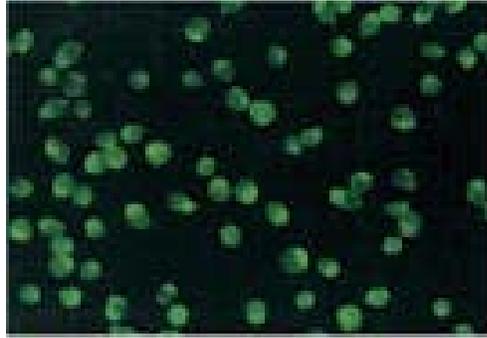
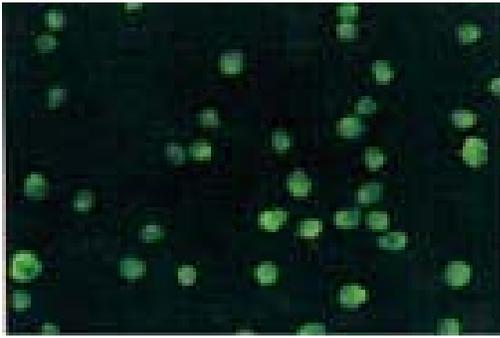
**Exhibit 3:** HIV-1 IFA Negative Control. Note that both the HIV-1 infected and uninfected control cells do not have any specific fluorescence and are essentially indistinguishable.



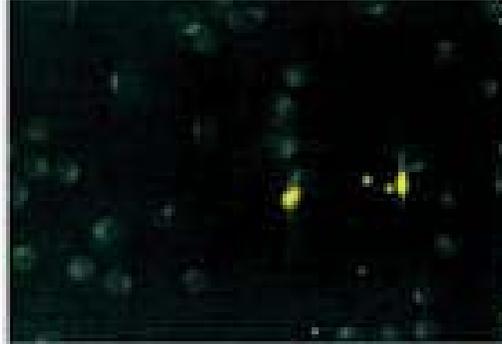
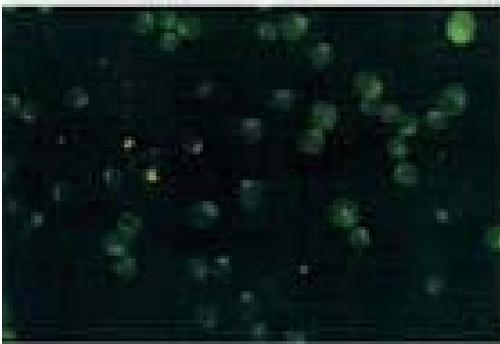
**Exhibit 4:** HIV-1 IFA Positive Control. Note the typical acentric apple-green fluorescent staining of the cytoplasm in the infected cells and the absence of fluorescein staining in the uninfected control cells.



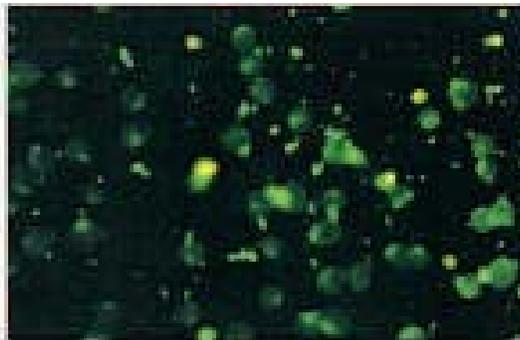
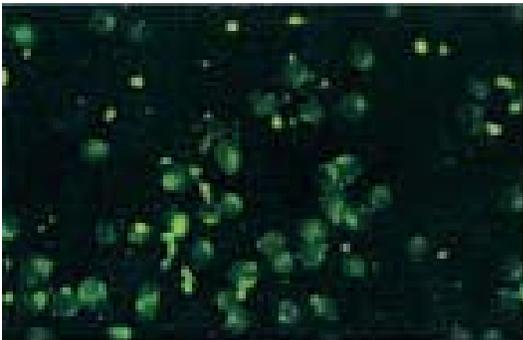
**Exhibit 5:** Strong positive staining reaction. Note that in the infected cell well only a fraction of the cells is actually stained.



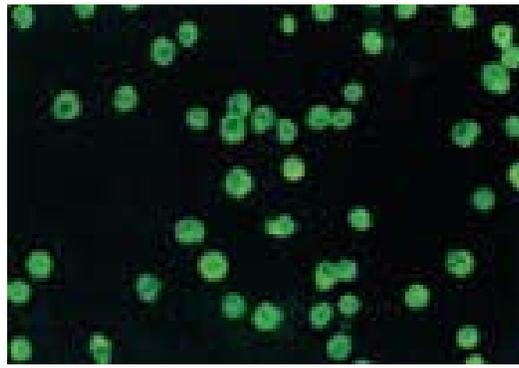
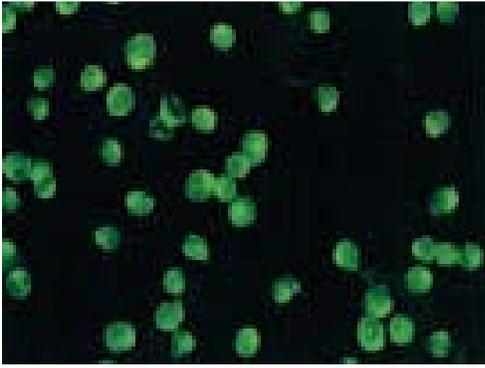
**Exhibit 6:** Very weak positive staining reaction. Note that some of the infected cells exhibit tiny specks of specific membrane fluorescence that is lacking in the uninfected control cells.



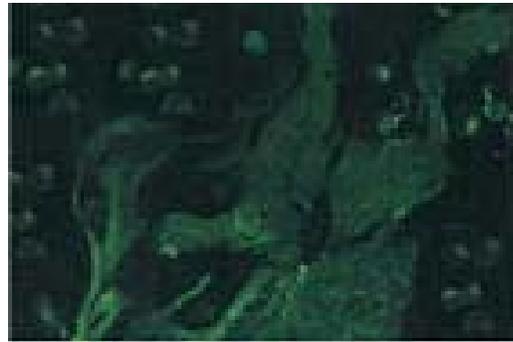
**Exhibit 7:** Negative reaction in the presence of extra cellular debris.



**Exhibit 8:** Negative reaction. Note the massive presence of mildly fluorescing debris of microbial or serum protein origin. A new specimen should be obtained if such an observation is made.



**Exhibit 9:** Non-specific staining reaction. This is on an SLE serum specimen displaying increased background fluorescence that is present on all infected or uninfected cells visible in the field of view.



**Exhibit 10:** Negative reaction in the presence of homogeneous extra cellular staining on the glass of the uninfected control cell well. This effect may occur with "aged" sera that have been stored refrigerated or at ambient temperature for prolonged periods.

**DESCRIPTION OF POSITIVE IFA RESULT:** Positive HIV-1 infected cells will exhibit a concentrated fluorescence in the cytoplasm of the cell. Because the PALL T-cell nucleus occupies a large portion of the cell volume, the cytoplasm tends to be compressed into a dense acentric structure, which is localized at one end of the cell. A positive cytoplasmic pattern can vary in appearance from an acentric "half moon" to a "polar cap", depending on the orientation of the T-cell fixed on the slide well. A positive pattern can range from diffuse to finely reticulated and the intensity can vary from a very intense brilliant apple-green color to a less intense apple-green color. The infected cell well contains 40-70% infected cells, often termed a "mixed cell" environment. Therefore, a positive specimen will exhibit specific fluorescence in at most 40-70% of the cells. The remaining cells are uninfected and serve as a built-in internal control and should have an appearance similar to the Negative Serum Control. **CAUTION: It is ALWAYS NECESSARY** to evaluate both the uninfected cell well and the infected cell well before interpreting the final IFA result. Be sure to scan a range of 3-5 microscopic fields within each cell well before completing the evaluation.

## INDETERMINATE RESULT INTERPRETATION CRITERIA

A test specimen is interpreted as **INDETERMINATE** when: 1) there is fluorescent staining present **IN BOTH** the HIV-1 infected and uninfected cells, or 2) when it is **NOT** possible to clearly differentiate the intensity of fluorescent staining and the pattern of fluorescence between the HIV-1 infected and uninfected cells, or 3) when duplicates are discordant. The test specimen is reported as **INDETERMINATE** and repeat testing of the original specimen should be carried out in duplicate. If an **INDETERMINATE** result persists, it may be necessary to obtain a fresh test specimen for follow up testing. The **INDETERMINATE** IFA interpretation does not imply that HIV-1 antibodies **are, or are not**, present in the test specimen. It simply means that the HIV-1 status of the serum or plasma specimen cannot be resolved through use of that specific Fluorognost HIV-1 IFA test run. **INDETERMINATE ASSAY RESULTS MUST NOT BE CONSIDERED POSITIVE OR NEGATIVE**. The correct evaluation in such situations must be based on subsequent repeat testing and/or immunoblot testing and clinical evaluation.

### DESCRIPTION OF INDETERMINATE IFA RESULT:

In most cases, **INDETERMINATE IFA** results are due to the presence of non-specific staining. In specimens with non-specific staining reactions, the fluorescence intensity can vary from very weak to very intense and staining will be exhibited in **BOTH** the infected and uninfected cells. When the specimen reacts equally with both the infected and uninfected cells, the IFA interpretation result must be regarded as inconclusive and reported as **INDETERMINATE**. Non-specific staining can be categorized as cellular and/or extra cellular and can occur as a result of a variety of conditions and from a number of sources. Cellular and extra cellular staining can also occur in the same sample. The following is a brief description of typical non-specific staining reactions:

#### **Non-specific Cellular Staining**

Non-specific cellular staining can occur when antibodies from the test sera bind with non-HIV-1 protein in both the HIV-1 infected and uninfected control cells. For example, sera from patients with Systemic Lupus Erythematosus (SLE) can produce intense cell membrane staining without cytoplasmic staining. Sera that possess antinuclear antibodies (ANA) react with the nucleus but not the cytoplasm of the cells. Some sera stain the cytoplasm of the cells and often display an appearance of a "polar cap"

#### **Non-specific Extra cellular Staining**

Non-specific extra cellular staining can appear in a wide variety of patterns including an amorphous film, droplets, particulate matter, bacterial/fungal contamination and dead cells. This type of staining generally does not interfere with the interpretation of the IFA result as long as the positive or negative result criteria are fulfilled.

It is important to note that it is also possible for a test specimen to present specific HIV-1 staining in the presence of non-specific staining. A specimen for which the positive interpretation criteria are clearly fulfilled is reported as **POSITIVE**. For some specimens, the non-specific staining may mask the presence of specific HIV-1 staining and hence, those samples should be reported as **INDETERMINATE**. If the IFA reader does not have confidence in the IFA interpretation, the test result should be considered to be **INDETERMINATE** and follow up testing with the original specimen should be carried out as outlined above. Caution must be exercised with sera containing microbial contamination since bacteria/fungus may reduce or eliminate antibody titer to HIV-1 and lead to a false-negative interpretation.

## LIMITATIONS OF THE PROCEDURE

The Fluorognost HIV-1 IFA protocol and the criteria for interpretation of the Fluorognost HIV-1 IFA test results must be performed in strict accordance with these instructions to obtain accurate results. Insufficient data are available to support use of Fluorognost HIV-1 IFA with individual body fluid specimens other than serum or plasma specimens or Home Access HIV-1 Test System DBS specimens. Similarly, use of the Fluorognost HIV-1 IFA with pooled or processed plasma and products made from such pools has not been evaluated, and testing of such specimens is not recommended. **Use of this licensed test kit with specimens other than those specifically approved for use with this test kit may result in inaccurate test results.**

While no specific group of specimens prone to false positive or false negative results can be identified, **INDETERMINATE** Fluorognost HIV-1 IFA results can be expected to occur with a slightly higher frequency in specimens from patients with certain autoimmune diseases such as Systemic Lupus Erythematosus, or in severe paraproteinaemias as seen in certain types of leukemia. On the other hand, viral infections such as hepatitis, cytomegalovirus, and Epstein - Barr virus have been demonstrated to be without significant influence on the results of Fluorognost HIV-1 IFA. Hyperlipemic or extremely icteric test specimens may cause evaluation problems resulting from the precipitation of specimen components onto the IFA slide. In addition, specimens heavily contaminated with bacteria may show atypical background fluorescence and may cause proteolytic degradation of the HIV-1 antibody. The testing of these samples is not recommended and every attempt should be made to obtain another specimen without that characteristics. The intensity of the fluorescence observed in HIV-1 positive specimens does not bear a strict correlation to the antibody titer, or to the presence of antibody directed against particular HIV-1 antigens. Although a POSITIVE IFA for antibodies to HIV-1 usually indicates infection with the virus, a diagnosis of Acquired Immunodeficiency Syndrome, AIDS, can only be established on clinical grounds, provided that an individual meets the case definition of AIDS established by the Centers for Disease Control.

An individual with a POSITIVE IFA for antibodies to HIV-1 should be referred for medical evaluation which may include additional testing. The clinical implications of antibodies to HIV in an asymptomatic individual are not known. However, a large proportion of such individuals has detectable HIV in their blood cells, and may develop Immunodeficiency with the passage of time.

**DUE TO VARIATIONS IN TEST PERFORMANCE AND THE UNCERTAINTY ASSOCIATED WITH INDETERMINATE IFA RESULTS, IT IS RECOMMENDED THAT ALL INDETERMINATE SAMPLES BE RETESTED USING THE ORIGINAL SPECIMEN. A FRESH SPECIMEN MAY ALSO BE REQUESTED FOR FOLLOW-UP TESTING. THE CORRECT EVALUATION IN SUCH SITUATIONS MUST BE BASED ON SUBSEQUENT REPEAT TESTING AND IMMUNOBLOT TESTING AND CLINICAL EVALUATION.**

**FLUOROGNOST HIV-1 IFA CLINICAL STUDIES HAVE DEMONSTRATED THAT THERE IS A HIGH DEGREE OF ASSAY REPRODUCIBILITY AND THAT IFA READER DISAGREEMENTS ARE UNCOMMON. HOWEVER, IN THE EVENT THAT MORE THAN ONE READER PARTICIPATES IN THE INTERPRETATION OF THE IFA AND/OR A FINAL RESULT CANNOT BE CONFIDENTLY DETERMINED BY THE IFA READER (S), THE ASSAY MUST BE REPEATED USING THE ORIGINAL SPECIMEN.**

**A NEGATIVE IFA DOES NOT EXCLUDE THE POSSIBILITY OF INFECTION WITH HIV-1. ANTIBODY TESTING SHOULD NOT BE USED IN LIEU OF BLOOD DONOR EXCLUSION AND SELF-EXCLUSION PROCEDURES.**

## EXPECTED RESULTS

### Reactivity in Blood Donor Populations: Comparison of IFA Reactivity with a licensed HIV-1 EIA

10,082 randomly selected blood donors were studied in a blinded manner by Fluorognost HIV-1 IFA in parallel with a licensed HIV-1 EIA at three geographically distinct US sites as shown in Table 1.

**TABLE 1**

REACTIVITY OF FLUOROGNOST HIV-1 IFA COMPARED TO A LICENSED HIV-1 EIA IN RANDOM BLOOD DONOR POPULATIONS

	Licensed EIA		Fluorognost IFA	
	Negative	Initially Reactive	Negative	Positive
SITE1	3755	68	3807	16
SITE 2	3985	5	3990	0
SITE 3	2269	0	2269	0
TOTALS	10,009	73	10,066	16

The correlations between EIA and IFA results shown in Table 1 are provided in Table 2.

Samples reactive by either EIA or IFA were further tested by a licensed Western Blot (WB, Cambridge Biotech) and in some cases by an investigational RIPA. The criteria for a positive RIPA were the presence of an env band, and either gp 41, gp 120 or gp 160.

**TABLE 2**

CORRELATION OF FLUOROGNOST HIV-1 IFA AND A LICENSED HIV-1 EIA IN BLOOD DONOR POPULATIONS

		EIA	
		Initially Reactive	Negative
IFA	Positive	15 <sup>a</sup>	1 <sup>b</sup>
	Negative	58 <sup>c</sup>	10,008

a. 12/15 samples WB positive, 2/15 WB indeterminate,

b. WB negative, RIPA negative (IFA false positive)

c. 1/58 was WB positive, RIPA pos (IFA false negative), 34/58 were WB negative (EIA false initially reactive, 13/58 were WB indeterminate (21/23 WB indeterminate were RIPA negative, 2/23 WB indeterminate had no RIPA performed)

Among the 10,082 donors screened, the EIA was initially reactive (IR) in 73 cases, including 15 with a positive licensed HIV-1 Western blot or RIPA. Among the remaining 58 EIA reactive samples, 34 had a negative WB, 24 had an indeterminate Western blot, (RIPA negative in 21 cases, and RIPA not done in 3 cases). 62/73 samples with an initially reactive EIA were retested in duplicate and 23 were repeatedly reactive, including 9 of the samples confirmed as positive for antibodies to HIV-1.

In comparison, the IFA was positive in 16 cases including 14 with a positive Western blot or RIPA, one case with a negative Western blot and RIPA and one unresolved case with an indeterminate Western blot and no RIPA performed. The IFA was false positive in one case.

## Reactivity in populations with AIDS or at high risk for AIDS

The reactivity of the Fluorognost HIV-1 IFA was investigated in subjects diagnosed with AIDS or ARC, and in members of a high-risk group. The data are summarized in Table 3.

**TABLE 3**

DETECTION OF ANTIBODIES TO HIV-1 IN SAMPLES FROM PATIENTS WITH AIDS, AIDS-RELATED COMPLEX (ARC) AND MEMBERS OF A HIGH RISK GROUP

Clinical Group	Fluorognost HIV-1 IFA			
	Number Tested	Number Positive	Number Negative	Percent Positive
AIDS	410	410	0	100%
ARC	64	63	1 <sup>b</sup>	98.4 %
High Risk <sup>a</sup>	661	597 <sup>c</sup>	64	90.3 %

a. Includes IVDU's, homosexual and bisexual men, hemophilia patients, heterosexual contacts of known infected individuals, recipients of multiple transfusions HTLV-1 seropositive persons from the Caribbean basin, and persons exposed accidentally to HIV infected blood or body fluids. The samples selected for the study included 598 which were EIA reactive and 63 which were EIA negative.

b. EIA negative, WB negative and RIPA negative

c. 583/597 were WB positive or RIPA positive, 1/597 was WB indeterminate and RIPA indeterminate, 10/597 were WB indeterminate and RIPA was not done, 1/597 was WB negative and RIPA positive (unresolved discordance with WB), 2/597 had neither WB nor a RIPA done.

d. 11/64 were WB negative, 17/64 were WB indeterminate and RIPA negative, and 2/64 were WB indeterminate and no RIPA was done, 34/64 were EIA negative, with no further testing done.

Fluorognost HIV-1 IFA was positive in 410/410 (100%) of the patients diagnosed with AIDS. Fluorognost HIV-1 IFA was positive in 63/64 patients diagnosed with AIDS related complex: one sample was negative by IFA also negative by a licensed EIA, licensed WB and RIPA. 593/597 IFA positive samples in the high risk group were further tested by licensed WB or RIPA. Among those 583 (98%) were positive by WB or RIPA, including 1 sample which was WB positive and RIPA negative (discordant assay result); 1 sample WB positive and RIPA indeterminate, 10 samples were WB indeterminate and RIPA was not done.

## PERFORMANCE CHARACTERISTICS

### REPRODUCIBILITY

Reproducibility of Fluorognost HIV-1 IFA was studied at clinical sites using a coded panel of 6 samples (see Table 4) along with the kit positive and negative serum controls. Three readers at each of two sites tested triplicate samples using 3 kit lots. Each sample was tested 54 times by Fluorognost HIV-1 IFA. The study demonstrated 100% accuracy in replicate testing.

**TABLE 4**  
CHARACTERIZATION OF FLUOROGNOST HIV-1 IFA REPRODUCIBILITY PANEL <sup>a</sup>

IFA READING	EIA <sup>b</sup> SIGNAL/CUTOFF RATIO
Positive (Strong)	>7.5
Positive (Strong)	>7.5
Positive (Moderate)	3.7
Positive (Moderate)	3.0
Negative	<0.3
Negative	<0.3

- a. Positive panel members displayed all major bands in licensed Western blots (p17, p24, p31, gp41, p53, p55, p64, gp120/160).
- b. Each sample was tested using a previously licensed EIA for antibodies to HIV-1.

In the remainder of the clinical trial two readers independently scored each assay. Table 5 summarizes the inter reader variability which was observed. A retest by IFA was performed on each sample with discordant readings, and additional testing was done by EIA, WB and RIPA.

**TABLE 5**  
CORRESPONDENCE OF FLUOROGNOST HIV-1 IFA RESULTS OBTAINED INDEPENDENTLY BY TWO READERS

CLINICAL GROUP	NUMBER TESTED	NUMBER CONCORDANT READINGS (%)	NUMBER DISCREPANT READINGS(%)	RESULT of REPEAT IF ASSAY
DONORS	10,082	10,062 (99.8)	20 (0.02)	negative
AIDS	410	410 (100)	0 (0.0)	---
ARC	64	63 (98.4)	1 (1.6)	negative <sup>a</sup>
High Risk	661	658 (99.5)	3 (0.5)	positive <sup>b</sup>
TOTAL	11,217	11,193 (99.8)	24 (0.2)	

- a. EIA negative, WB negative, RIPA negative
- b. EIA positive, WB positive

Overall, the concordance of interpretation of the Fluorognost HIV-1 IFA was 99.78% when the two readers independently scored the same IFA slides. For three of the discordant readings there were false negative results by a single reader. The EIA signal to cutoff ratios for these samples were 6.6, 5.6, and 2.9. For 21 of the discordant readings there were false positive results by a single reader.

## SPECIFICITY

### Specificity in Medical Conditions Unrelated to HIV-1

Fluorognost HIV-1 IFA reactivity was studied in the serum of patients with medical conditions unrelated to HIV-1 infection (Table 6). All 395 samples tested by Fluorognost HIV-1 IFA gave negative results

**TABLE 6**  
REACTIVITY OF FLUOROGNOST HIV-1 IFA IN SERUM OF PATIENTS WITH MEDICAL CONDITIONS UNRELATED TO HIV-1 INFECTION

MEDICAL GROUP	NUMBER STUDIED
HTLV-1	31
Epstein-Barr Virus	17
Other Viral Diseases <sup>a</sup>	45
Non-viral Diseases <sup>b</sup>	44
Leukemias <sup>c</sup>	144
Rheumatoid Arthritis	47
Systemic Lupus Erythematosus	19
CNS Degenerative Diseases <sup>d</sup>	7
Lipemia	30
Elevated Bilirubin	10

- a. Includes 18 cytomegalovirus, 2 hepatitis A, 24 hepatitis B and 1 herpes zoster.
- b. Includes 5 *Borrelia* (lyme disease), 16 *E. coli*, 2 *Salmonella*, 3 *Streptococcus*, 6 *Syphilis* and 12 toxoplasmosis infections.
- c. Includes 58 chronic lymphocytic leukemia (CLL), 15 acute lymphocytic leukemias (ALL), 17 chronic myeloid leukemias (CML), 41 acute myeloid leukemias, and 13 pre-leukemic myelodysplastic syndrome (MDS).
- d. Includes 4 multiple sclerosis and 2 Alzheimer's disease.

### Specificity for Screening in Low Risk Populations

Specificity was determined for screening in a low risk population of blood donors. Based on the data presented in Tables 1 and 2, the specificity of the Fluorognost HIV-1 IFA was 99.98% (two false positive tests out of 10,067 negative samples screened). In comparison, the specificity of the EIA, as determined in the same clinical trial, was 99.95% (14 false positive tests out of 10,062 negative samples screened). Specificity was calculated with the following formula:

$$\frac{(\text{Number Tested}^a) - (\text{Number Reactive}^b)}{(\text{Number Tested}^a) - (\text{Number Positive by WB or RIPA})}$$

- a. For EIA, calculations excluded samples which were initially reactive, but failed to be retested in duplicate.
- b. For IFA, "reactive" means positive on a single determination. For EIA, "reactive" means repeatedly reactive.

The number of false positive results obtained with the Fluorognost HIV-1 IFA was significantly less than the number of false positive results obtained with EIA,  $p < 0.001$ . Statistical analysis was based on repeatedly reactive EIA results using McNemar's test with one degree of freedom and two-sided p-value.

These data suggest increased specificity of the Fluorognost HIV IFA compared to the EIA in blood screening of low risk populations.

### Specificity for Additional Testing of EIA Repeatedly Reactive Samples

The specificity of Fluorognost HIV-1 IFA was compared with a licensed Western blot (Cambridge Biotech) for additional testing of samples found to be repeatedly reactive by EIA in different clinical groups, including blood donor populations. Unresolved or discrepant samples were further tested by an investigational RIPA assay, as previously described.

Table 7 summarizes a comparison of Fluorognost HIV-1 IFA results with licensed Western blot results for EIA reactive blood donor samples. The data presented in Table 7 include the 73 blood donor samples found in the prospective study reported in Table 1. In addition, the table contains data from 155 EIA repeatedly reactive samples which were collected sequentially on referral from blood donor screening sites, and studied at a single clinical site.

**TABLE 7**

COMPARISON OF RESULTS OF FLUOROGNOST HIV-1 IFA WITH A LICENSED WESTERN BLOT FOR EIA REACTIVE SAMPLES FROM DONOR POPULATIONS

IFA	WESTERN BLOT		
	Positive	Indeterminate	Negative
Positive	61	9 <sup>a</sup>	0
Negative	1 <sup>b</sup>	70 <sup>c</sup>	87

- a. 6/9 were RIPA POS, 2/9 were RIPA NEG; 1/9 no RIPA was done
- b. RIPA POS (IFA false negative)
- c. 68/70 were RIPA NEG; 2/70 no RIPA was done

Fluorognost HIV-1 IFA was highly concordant with the Western blot: 61/62 (98.4%) of the samples with positive blots and 87/87 (100%) of the samples with negative blots. The IFA was falsely negative in one case, as previously reported in Table 2.

Fluorognost HIV-1 IFA was also compared with a licensed Western blot on EIA reactive samples primarily from AIDS and high risk populations. These data, summarized in Table 8, include the AIDS, ARC and high risk group samples reported in Table 3 plus 21 additional samples from persons with unknown risk factors.

**TABLE 8**  
**COMPARISON OF FLUOROGNOST HIV-1 IFA WITH A LICENSED WESTERN BLOT IN CLINICAL GROUPS INCLUDING AIDS, ARC, AND HIGH RISK**

IFA	WESTERN BLOT		
	Positive	Indeterminate	Negative
Positive	917	141 <sup>a</sup>	1 <sup>b</sup>
Negative	1 <sup>c</sup>	14 <sup>d</sup>	13

- a. 124/141 samples were RIPA POS; 16/141 samples had no RIPA; 1/141 samples was RIPA IND
- b. RIPA POS sample (unresolved discordance with WB)
- c. RIPA NEG sample (unresolved discordance with
- d. 13/14 samples were RIPA NEG; 1/14 samples had no RIPA

From the clinical study results provided in Tables 7-8, the specificity of the Fluorognost HIV-1 IFA on EIA reactive samples was estimated to be 99.0% compared with the Western blot (100 samples IFA negative among 101 Western blot negatives). This demonstrates equivalent specificity of the IFA compared with the Western blot as an additional test on EIA reactive samples.

### Resolution of EIA Reactive Samples with an Indeterminate Western Blot

Among the 234 samples with an indeterminate Western blot shown in Tables 7 and 8, RIPA was performed on 214 samples (91.5%) and resolution was obtained in 213 cases. Fluorognost HIV-1 IFA was concordant with RIPA for all 130 RIPA positive and 81 of 83 RIPA negative samples. These data suggest that the IFA may be useful in resolving samples with an indeterminate Western blot result.

For the same set of samples, the IFA result was compared to the Western blot result that would have been obtained if the blot had been interpreted according to the published criteria of the U.S. Public Health Service (CDC. Interpretation and use of the Western blot assay for serodiagnosis of human immunodeficiency virus type 1 infection. MMWR 1989; 38(S-7): 1 -7.) This analysis is shown in Table 9.

**TABLE 9**

CORRELATION OF FLUOROGNOST HIV-1 IFA RESULTS WITH WESTERN BLOT REACTIVITY  
FOLLOWING REINTERPRETATION OF 234 WESTERN BLOT INDETERMINATE SAMPLES USING  
PUBLISHED CRITERIA OF THE U.S. PUBLIC HEALTH SERVICE

Clinical Group	IFA Result	Number of Samples Reinterpreted	
		Western Blot Positive	Western Blot Indeterminate
Blood Donors	Positive	4	5
	Negative	3	67
AIDS, ARC, High Risk and Unknown Risk	Positive	78	63
	Negative	6	8

As shown in Table 9, a significant proportion of samples with a positive IFA would have been interpreted as concordant with a positive Western blot by the revised criteria (82/150, 54.7%). A smaller proportion of samples with negative IFA results would have been discordant with positive western blot results (9/84, 10.7%). Of the latter 9 samples, eight were tested by RIPA and found negative.

## **SENSITIVITY**

### **Sensitivity of Fluorognost HIV-1 IFA**

Sensitivity of Fluorognost HIV-1 IFA was estimated to be 100% based on identification of HIV-1 antibodies in 402 of 402 patients with a clinical diagnosis of AIDS (Table 3).

In non-AIDS groups, based on the clinical studies summarized in Tables 7-8, a total of 709 EIA reactive samples had a positive Western blot or RIPA. Among these, the IFA was positive in 707 cases giving an estimated sensitivity of 99.7% compared with the EIA, establishing equivalence.

Of the two cases with a possible false negative IFA, one case, from a person with unknown risk, had discrepant results by Western blot and RIPA. The second case occurred in blood donor screening. In this case, the EIA sample to cut-off ratio was 4.1 and the Western blot was positive for all major bands. The possibility of an operator error (failure to add the sample in the IFA test) could not be excluded.

### **Reactivity of Fluorognost HIV-1 IFA in EIA Repeatedly Reactive Samples from Geographically Diverse Areas**

290 samples from geographically distinct areas were examined by Fluorognost HIV-1 IFA in a blinded study conducted at two independent sites. The samples, which were repeatedly reactive by a licensed EIA, included 77 samples from Zaire, 39 samples from Brazil, 24 samples from Belgium and 150 samples from Austria. 272 of these samples were positive by licensed Western blot and 18 were indeterminate. All 290 samples tested positive by IFA. Sensitivity of Fluorognost IFA for HIV-1 was 100% for the 272 Western blot positive samples in this set of international samples.

## **APPENDIX SECTION**

### **TECHNICAL ASSISTANCE**

**If any questions or problems arise regarding the evaluation and interpretation of IFA results please contact a Sanochemia representative provided on the back cover. Phone (1) 203 564 1580 and 0044 (0) 117 929 0287.**

## TROUBLESHOOTING GUIDE

### POSITIVE CONTROL DOES NOT REACT WITH INFECTED CELLS

#### POSSIBLE CAUSE

- Wrong serum control used
- Serum not diluted properly
- FITC-Conjugate not diluted properly
- Deterioration of IFA slide

#### REMEDY

- Repeat test with fresh POS serum control
- Check serum dilution.
- Check Conjugate dilution.
- Check for presence of antigen in slides with other positive sera.

### NEGATIVE CONTROL REACTS WITH ANTIGEN OR WITH ALL CELLS

#### POSSIBLE CAUSE

- Wrong serum used
- Serum not diluted properly
- Serum contaminated with a Positive Serum Control
- Serum or Conjugate allowed to dry in cell wells
- Negative Serum Control or FITC-Conjugate contaminated

#### REMEDY

- Repeat test.
- Check Serum and FITC-Conjugate Dilutions.
- Check morphology of staining reaction to see if it is typical of an HIV-2 Positive Serum Control or a Non-specific reaction.
- Do not allow the specimen or conjugate to dry in the well. Use a humidity chamber during incubation.
- Use a fresh aliquot of the original Negative Serum Control.

### DISTORTION OF OPTICAL IMAGES

#### CAUSE

- Not enough Mounting Media
- Too much Mounting Media
- Air Bubbles under the Cover slip

#### REMEDY

- Place one drop of Mounting Media in each well. The Mounting Media must fill the space between the cell well and the Cover slip. Carefully remove the Cover slip, add more Mounting Media, and remount.
- Excess Mounting Media can interfere with the optical path and distort the image. Do not try to wipe off excess fluid; float the Cover slip off with PBS Diluent and use a fresh Cover slip.
- Gently press down on the glass Cover slip without forcing Mounting Media from between the glass layers.

## UNEVEN FLUORESCENCE

### CAUSE

- Specimen dried to cell well
- Inadequate volume of specimen in cell well
- Cell Well contamination from adjacent wells

### REMEDY

- Do not allow the cell wells to dry after specimen and conjugate addition. Be sure to use a humidity chamber during incubation.
- Apply 15 µl of specimen to each cell well making sure that the entire cell well is covered. Repeat the test.
- If the cell well is overfilled with specimen, the slide is tilted during handling, or not placed on a level surface, there is potential for cross-contamination. Repeat the test.

## LOW INTENSITY FLUORESCENCE

### CAUSE

- Improper storage or shipping of test reagents
- Improper dilution of FITC-Conjugate
- Serum specimen not diluted properly
- Gross contamination of test sera

### REMEDY

- Check expiration date of Fluorognost reagents, shipping receipt records, and storage conditions.
- Make sure FITC-Conjugate is diluted properly with PBS Diluent prior to use.
- Use 1:30 dilution with PBS Diluent solution using sterile distilled water or water purified by reverse osmosis.
- Store specimens at 2-8 degrees C up to 7 days, store at -20 degrees C for longer periods of time. Try to obtain fresh sample. Filter as last resort.

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