

# ANTIBODY TO HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 p24 ANTIGEN (MURINE MONOCLONAL)

## COULTER HIV-1 p24 Antigen Assay FOR IN VITRO DIAGNOSTIC USE

### NAME AND INTENDED USE

The COULTER HIV-1 p24 Antigen Assay is a qualitative and quantitative in vitro Enzyme Immunoassay (EIA, or Enzyme-linked Immunosorbent Assay, ELISA) for the detection of uncomplexed p24 antigen of the Human Immunodeficiency Virus Type 1 (HIV-1) in human plasma or serum, or tissue culture supernatants. It is intended to be used for screening blood donors or other individuals at unknown risk for HIV-1 infection, and as an aid in diagnosis of HIV-1 infection and prognosis or monitoring of disease progression.

The COULTER HIV-1 p24 Antigen Assay is intended to be used with the reagents supplied with the COULTER HIV-1 p24 Antigen Neutralization Kit (sold separately) as a qualitative, additional, more specific test for the detection of HIV-1 p24 antigen in plasma, serum, or tissue culture supernatants.

### SUMMARY AND EXPLANATION OF THE TEST

The Human Immunodeficiency Virus Type 1 (HIV-1) is recognized as the etiologic agent of acquired immunodeficiency syndrome (AIDS). The virus is transmitted by sexual contact, exposure to infected blood, certain body fluids or tissues, and from mother to fetus or child during the perinatal period.<sup>1,6</sup> After exposure to the virus, HIV-1 infection is characterized by an early period of antigenemia in which HIV-1 antigens are detectable in blood.<sup>8,9,10,11</sup> One of the viral antigens present in blood during antigenemia is the core protein, p24, the major internal structural protein of HIV-1. For some infected people, this period of early antigenemia precedes the appearance of detectable HIV antibodies in blood (seroconversion) by days or weeks.<sup>12,15</sup> As virus-specific antibodies are produced, they complex with free antigens so that the level of free antigens in the circulation tends to decline, or disappear during the asymptomatic period of the disease.<sup>15,16</sup> Patients may remain asymptomatic for weeks or years, but as the disease progresses, patients develop clinical symptoms and eventually progress to AIDS.<sup>17,20</sup> During the later stages of the disease, circulating antibody levels tend to decline and characteristically, there is another period of antigenemia.<sup>4</sup> The COULTER HIV-1 p24 Antigen Assay is an enzyme immunoassay (EIA, or Enzyme-linked Immunosorbent Assay, ELISA) developed for detection and quantitation of the HIV-1 p24 core protein. COULTER HIV-1 p24 Antigen Neutralization Kit (sold separately) is a qualitative, supplemental assay used with the antigen assay as an additional assay to confirm the presence of HIV-1 p24 antigen in clinical specimens.

Direct evidence for the presence of infectious virus in a specimen is routinely obtained by co-culturing specimens with target cells that are susceptible to infection by HIV-1, such as immortalized human monocytes, T-cells, or human peripheral blood lymphocytes. The detection and quantitation of HIV-1 p24 antigen in tissue culture supernatants has been used to monitor and characterize replication of the virus.<sup>21</sup>

### PRINCIPLE OF THE PROCEDURE

The COULTER HIV-1 p24 Antigen Assay uses a murine monoclonal antibody to HIV-1 p24 antigen coated onto microtiter strip wells. Purified HIV-1 p24 antigen reagent derived from a human T-cell line infected with the HTLV-IIIB strain of HIV-1 is used as a quantitative calibration standard. A specimen of plasma, serum, or tissue culture media and lyse buffer are added to a coated well and incubated. Lyse buffer disrupts virus particles in a specimen and HIV-1 p24 antigen, if present in the specimen, binds to the monoclonal antibody of the microtiter well. Following a wash step, biotinylated human anti-HIV-1 IgG is added to the well and, during incubation, binds to any HIV-1 p24 antigen bound to the well. Following another wash, streptavidin-horseradish peroxidase is added which complexes with biotinylated antibodies. In a final step, a substrate reagent containing tetramethylbenzidine (TMB) and hydrogen peroxide is added which reacts with complexed peroxidase to form a blue color. The reaction is terminated by the addition of acid, COULTER Stopping Reagent (CSR-1), and the absorbance is measured spectrophotometrically. The intensity of the color development is directly proportional to the amount of uncomplexed p24 antigen in the plasma, serum, or tissue culture media. The presence of HIV-1 p24 antigen in a specimen is qualitatively indicated if its absorbance is greater than or equal to the assay cutoff value. The quantity of free HIV-1 p24 antigen in a specimen may be determined by comparing its absorbance with that of purified HIV-1 p24 antigen Calibration Standards.

Specimens that are repeatedly reactive in the COULTER HIV-1 p24 Antigen Assay must be tested using the COULTER HIV-1 p24 Antigen Neutralization Kit (sold separately). The neutralization assay is a qualitative test that uses the principle of specific antibody-antigen complex formation to reduce the amount of free antigen available for binding in the COULTER HIV-1 p24 Antigen Assay. A specimen is incubated with purified human anti-HIV-1 IgG (Neutralizing Reagent) in an antibody-coated microtiter well. In parallel, a duplicate specimen is incubated with normal human globulins (Negative Neutralizing Control) in a second coated well and the test is performed in both wells as described above. If HIV-1 p24 antigen is present in a specimen, the color development is reduced by at least 40% in the presence of the Neutralizing Reagent compared with the Negative Neutralizing Control.

### REAGENTS

	96-well
1. Antibody to HIV-1 p24 antigen (Murine Monoclonal) Coated Microtiter Plate (HIV Reaction Plate)	1 x 96 wells
2. HIV-1 p24 Antigen Reagent (Lyophilized; contains sodium azide, 0.1%)	2 vials
3. Anti-HIV (human)-Biotin Reagent (lyophilized; preservative - thimerosal, 0.2%)	1 vial
4. Normal Human Serum [NHS, Negative for HIV-1 antigen(s), anti-HIV-1 and 2, HBsAg, and anti-HCV; contains sodium azide, 0.1%]	6.0 mL
5. SA-HRPO (Streptavidin conjugated to horseradish peroxidase; gentamicin, 0.01% and thimerosal, 0.1%)	0.1 mL
6. SA-Buffer (Tris buffer for SA-HRPO; preservatives - gentamicin, 0.01% and thimerosal, 0.1%)	21.0 mL
7. TMB Reagent in Dimethylsulfoxide	0.5 mL
8. TMB Diluent (contains citrate phosphate buffer, 0.0045%, H <sub>2</sub> O <sub>2</sub> and 2-chloroacetamide, 0.1%)	21.0 mL
9. Lyse Buffer (contains Triton X-100, dipotassium EDTA, Tween-20; preservative - thimerosal, 0.05%)	4.5 mL
10. Wash Buffer (contains potassium phosphate buffer, 20X Conc., Tween-20, and 2-chloroacetamide, 2.0%)	75.0 mL
11. CSR-1 (4N H <sub>2</sub> SO <sub>4</sub> )	5.0 mL
12. Uncoated Strip(s)	1
13. Plate Covers	4 sheets

## REAGENTS

	2400-well
1. Antibody to HIV-1 p24 antigen (Murine Monoclonal) Coated Microtiter Plate (HIV Reaction Plate)	25 x 96 wells
2. HIV-1 p24 Antigen Reagent (Lyophilized; contains sodium azide, 0.1%)	50 vials
3. Anti-HIV (human)-Biotin Reagent (lyophilized; preservative - thimerosal, 0.2%)	25 vials
4. Normal Human Serum [NHS, Negative for HIV-1 antigen(s), anti-HIV-1 and 2, HBsAg, and anti-HCV; contains sodium azide, 0.1%]	30 x 6.0 mL
5. SA-HRPO (Streptavidin conjugated to horseradish peroxidase: gentamicin, 0.01% and thimerosal, 0.1%)	25 x 0.1 mL
6. SA-Buffer (Tris buffer for SA-HRPO; preservatives - gentamicin, 0.01% and thimerosal, 0.1%)	25 x 21.0 mL
7. TMB Reagent in Dimethylsulfoxide	25 x 0.5 mL
8. TMB Diluent (contains citrate phosphate buffer, 0.0045%, H <sub>2</sub> O <sub>2</sub> and 2-chloroacetamide, 0.1%)	25 x 21.0 mL
9. Lyse Buffer (contains Triton x100, dipotassium EDTA, Tween-20; preservative - thimerosal, 0.05%)	25 x 4.5 mL
10. CSR-1 (4N H <sub>2</sub> SO <sub>4</sub> )	25 x 5.0 mL
11. Plate Covers ( <u>Shipped separately</u> )	200 sheets

**NOTE: The following product is sold separately by COULTER CORP.:**  
Wash Buffer (20X Concentrate) P/N 6607052

## WARNINGS AND PRECAUTIONS

### Safety Warnings

Handle all biological materials in the COULTER HIV-1 p24 Antigen Assay and the COULTER HIV-1 p24 Neutralization Kit as though capable of transmitting infectious agents. The Biotin, Normal Human Serum (NHS) and HIV-1 p24 Antigen Reagent have been inactivated. Test methods are not known, however, that can offer assurance that products derived from human blood will not transmit infection.

Handle all biological specimens, including tissue culture materials, as though capable of transmitting infectious agents. We recommend that biological materials be handled according to established Good Laboratory Practices (GLP) guidelines, Occupational Safety and Health Administration (OSHA) guidelines, and the Centers for Disease Controls and Prevention (CDC) guidelines for working with HIV material in the United States of America. In all other countries, we recommend following guidelines and recommendations equivalent to those of the CDC, OSHA, and GLP.

- Dispose of all materials that have come into contact with specimens and reagents in accordance with local, state and federal regulations. Solid wastes may be incinerated or autoclaved for an appropriate period of time. Due to variations among autoclaves and in waste configuration, each user must verify the effectiveness of this decontamination cycle using biological indicators. Do not autoclave materials containing bleach.
- Laboratory coats are recommended for all personnel handling kit reagents and specimens. Laboratory coats should be removed before leaving the laboratory and autoclaved before disposal to assure containment.
- Disposable gloves should be worn while handling potentially infectious materials, including sample tubes. Hands should be washed with an effective anti-microbial skin cleanser after handling these specimens.
- Spills involving non-acidic liquids should be wiped promptly and thoroughly with a 0.5% solution of sodium hypochlorite, or other effective disinfectant, such as 0.5% Wescodyne<sup>22,23</sup> to decontaminate the area. Spills involving acidic liquids should be wiped dry first and then the area should be wiped with a 0.5% solution of sodium hypochlorite to decontaminate. Materials used to wipe up spills should be treated as hazardous waste.
- Use mechanical devices for pipetting. Do not pipet by mouth. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
- Some of the reagents in this kit contain sodium azide. Sodium azide under acidic conditions yields hydrazoic acid, an extremely toxic compound. After decontamination, if azide compounds are disposed of in a plumbing system, they should be diluted and flushed with generous amounts of running water. These precautions are recommended to avoid the accumulation of deposits in metal piping in which explosive conditions could develop.
- Do not pour bleach and CSR-1 (4N H<sub>2</sub>SO<sub>4</sub>) in sink at the same time.
- All waste from plate washer should be disposed of as hazardous waste.

### Precautions

- The COULTER HIV-1 p24 Antigen Assay and COULTER HIV-1 p24 Antigen Neutralization Kit are for in vitro diagnostic use only.
- Do not mix reagents from different kits.
- Do not use this kit beyond the expiration date shown on the package label.
- The COULTER HIV-1 p24 Antigen Neutralization Kit is to be used only with the COULTER HIV-1 p24 Antigen Assay.
- Because of the instability of the analyte, all samples should be tested for HIV-1 antigen as soon as possible after they are drawn, or else promptly stored at -20°C pending testing. Where operational conditions preclude rapid testing or frozen storage, signal loss can be minimized by limiting pre-test storage at room temperature (not exceeding 26°C) and refrigeration (nominal 4°C) to a maximum of 7 days, including no more than three days at room temperature. Samples for which testing cannot be completed within 7 days may be stored frozen at -20°C +/- 2°C or -70°C +/- 5°C, for a maximum of three years prior to testing.
- Protect all kit components from exposure to bleach or bleach residue (white powder) that may remain from decontaminating hands and work surfaces.
- Procedures that generate aerosols should be avoided.
- Do not reuse coated strips or partially used coated strips, since this may cause erroneous test results.
- Do not reuse plate covers.
- The use of disposable pipet tips is recommended.
- Distilled or deionized water must be used for reconstitution of reagents and preparation of Wash Buffer. Clinical laboratory reagent grade water Type I or Type II is acceptable.<sup>24</sup> Store water in nonmetallic containers.

## PREPARATION OF REAGENTS

Bring all reagents to room temperature (15-30°C) prior to use. Store reconstituted reagents and unused reagents at 2-8°C, unless otherwise noted.

### HIV-1 p24 Antibody-coated Microtiter Strips

1. Bring pouch containing HIV-1 p24 antibody-coated microtiter strips (HIV Reaction Plate) to room temperature (15-30°C) before opening to avoid condensation on the strips.
2. The plate consists of 12 removable strips of 8 wells each. Any partial use of a strip commits all 8 wells to the assay. Antibody-coated strips may be used only once. When using a 96-well plate washer and fewer than 12 strips are needed, place uncoated strips in the remaining positions.
3. Unused strips may be placed back into the pouch and sealed with the desiccant provided and stored at 2-8°C for 60 days.

### Wash Buffer Working Dilution

1. Prepare 700mL of Wash Buffer Working Dilution by diluting 35mL of the 20X Wash Buffer with 665mL of distilled water.  
**Note:** When a 96-well plate is used, about 700mL of the Wash Buffer Working Dilution is required to perform the washing steps. Discard any unused reagent at the end of the day.

### Reconstitution of HIV-1 p24 Antigen Reagent

1. Add 0.5mL of water to the HIV-1 p24 Antigen Reagent vial and recap the vial.
2. Gently invert the vial to mix contents. Allow 5 minutes for contents to dissolve. (The reconstituted Antigen Reagent has a concentration of HIV-1 p24 antigen of 625 pg/mL)
3. The reconstituted reagent is stable for 2 weeks when stored at 2-8°C. After reconstituting, determine the expiration date and write this date on the vial label.  
**Note: Each Positive Control well requires 50µL of the reconstituted Antigen Reagent.**

### Preparation of Diluted Positive Control (Alternate Method)

1. The Positive Control may be pre-diluted as an alternative to preparing the Positive Control in the assay well as described under Setup Procedures.
2. Add 150µL of reconstituted HIV-1 p24 Antigen Reagent to 600µL of Normal Human Serum (NHS).
3. Diluted Positive Control is stable for eight hours at room temperature (15-30°C) prior to use in the assay.  
**Note: Each Positive Control well requires 250µL of the Diluted Positive Control.**

### Reconstitution of Biotin Reagent

1. Add 21mL of water to the Biotin Reagent vial and recap the vial.
2. Gently invert vial to mix contents. Allow 5 minutes for the contents to dissolve.
3. The reconstituted reagent is stable for 2 months when stored at 2-8°C. After reconstituting, determine the expiration date and write this date on the vial label.  
**Note: Each well requires 200µL of the reconstituted Biotin Reagent.**

### SA-HRPO Working Dilution

**Caution: SA-HRPO Working Dilution should be prepared within eight hours of its use.**

1. To prepare the SA-HRPO Working Dilution for a complete 96-well plate, add 21L of SA-HRPO reagent to 21 mL of SA-Buffer. Mix well and use.
2. If a partial plate is used, prepare enough SA-HRPO Working Dilution as shown below:

No. of Tests	SA-Buffer (mL)	SA-HRPO (µL)
24	5.0	5
48	10.0	10
72	15.0	15

**Note:** Each well requires 200µL of the SA-HRPO Working Dilution.

### TMB-Substrate Solution

**Caution: TMB-Substrate Solution should be prepared within 1 hour of use.**

1. To prepare the TMB-Substrate Solution for a complete 96-well plate, pipette 21mL of the TMB Diluent provided in the kit into a clean disposable plastic container and add 210µL of TMB Reagent. Mix well and use.
2. If a partial plate is used, prepare enough TMB-Substrate Solution as shown below:

No. of Tests	TMB Diluent (mL)	TMB Reagent (µL)
24	5.0	50
48	10.0	100
72	15.0	150

**Note:** TMB-Substrate Solution should appear colorless and, when combined with CSR-1 solution, should have an absorbance value less than 0.050 at 450 nm or 450/570 nm when compared with a distilled water blank.

Each well requires 200µL of the TMB-Substrate Solution.

## STORAGE

When not in use, store the kit and all components at 2-8°C, except the Wash Buffer which may be stored at 2-30°C. Store reconstituted reagents at 2-8°C.

## CHEMICAL AND PHYSICAL INDICATIONS OF INSTABILITY

Alteration in the physical appearance of test kit material may indicate instability or deterioration.

TMB-Substrate Solution should appear colorless. Appearance of a light blue color indicates deterioration, and the TMB-Substrate Solution should not be used.

## SPECIMEN COLLECTION, PREPARATION AND STORAGE

1. Plasma collected in acid-citrate-dextrose (ACD), citrate-phosphate-dextrose with adenine (CPDA-1), EDTA, sodium citrate or heparin, or serum may be used and should be tested as soon as possible following collection. No special preparation of the patient is required prior to blood collection.

- Because of the instability of the analyte, all samples should be tested for HIV-1 antigen as soon as possible after they are drawn, or else promptly stored at -20°C pending testing. Where operational conditions preclude rapid testing or frozen storage, signal loss can be minimized by limiting pre-test storage at room temperature (not exceeding 26°C) and refrigeration (nominal 4°C) to a maximum of 7 days, including no more than three days at room temperature. If the initial EIA, the repeat EIA, and the neutralization test cannot be completed within 7 days, the specimens may be stored frozen at -20°C +/- 2°C or -70°C +/- 5°C for up to 3 years. The effect of longer storage on the stability and detectability of HIV-1 p24 antigen is unknown.
- Remove the serum from the clot or plasma from the red cells as soon as possible to avoid hemolysis. COULTER HIV-1 p24 Antigen Assay and COULTER HIV-1 p24 Neutralization assay are not affected by elevated hemoglobin up to 350 mg/dL or triglycerides up to 474 mg/dL.
- Specimens containing particulate matter may give inconsistent test results. Such specimens should be clarified by centrifugation prior to assay.
- Avoid subjecting specimens to repeated freeze/thaw cycles. Studies show that accurate test results can be obtained for specimens subjected to five freeze/thaw cycles. Studies show that accurate test results can be obtained for specimens subjected to as many as five freeze/thaw cycles. More than five freeze/thaw cycles may cause inaccurate results.
- Plasma and serum specimens should be used without dilution.
- Bring all specimens to room temperature (15-30°C) prior to assay.

### HIV-1 P24 ANTIGEN ASSAY PROCEDURES

#### Materials Supplied

COULTER HIV-1 p24 Antigen Assay, 96 (PN 6604535) or 2400 (PN 6607051) test kits. See Reagents section of this package insert for a complete listing.

#### Materials Required But Not Provided

- COULTER Wash Buffer, PN 6607052 (4 x 1.9L), is sold separately, for use with the COULTER HIV-1 p24 Antigen Assay, PN 6607051
- COULTER HIV-1 p24 Antigen Neutralization Kit, PN 6604536 (required for specimens that are repeatedly reactive in the COULTER HIV-1 p24 Antigen Assay)
- Deionized or distilled water, Type I or II Clinical Laboratory Reagent Grade Water
- Micropipetting devices capable of delivering 10µL, 20µL, 50µL, 200µL volumes and suitable disposable tips
- Incubator without CO<sub>2</sub>, capable of maintaining 37°C +/- 2°C
- Latex gloves
- 5% Hypochlorite solution (household bleach) or appropriate disinfectant
- Timer
- Graduated cylinders and beakers
- Microplate Washer, Coulter PN 2907112, or equivalent, microelisa plate washer with waste trap and vacuum source
- Microplate Reader, Coulter PN 2907117, or equivalent, microelisa plate reader or dual wavelength spectrophotometer capable of measuring absorbance at 450 nm with reference at 570 nm is preferred.
- Single wavelength spectrophotometer capable of measuring absorbance at 450 nm may be used. Instruments used to determine absorbance should have a linear range of 0 to 2.500 optical density units. Consult the instrument manufacturer's specifications.
- Computer System, and software, Coulter PN 2907110 and PN 2907113, or equivalent

#### Preliminary Procedural Considerations

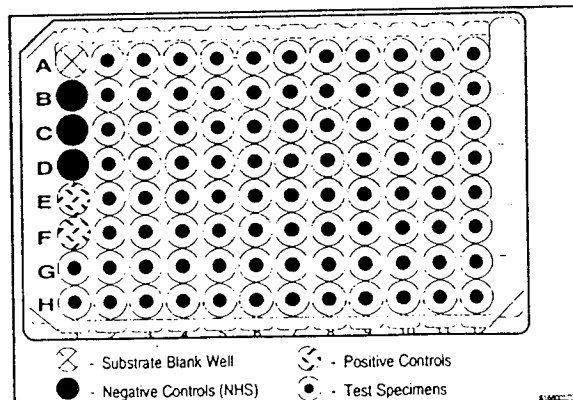
- The testing area should be separate from areas where blood or blood products for transfusion are stored.
- Use only reagents from the same kit lot. Materials should not be used after the expiration date shown on the package or vial label. Materials and specimens should be at room temperature (15-30°C) before testing begins.
- The COULTER HIV-1 p24 Antigen Assay and COULTER HIV-1 p24 Neutralization assay require efficient washing of the microtiter wells at various steps of the assay procedures using an ELISA Microplate Washer. It is important to follow the directions provided with your washer. To wash the microtiter wells efficiently, allow wells to soak in the Wash Buffer Working Dilution for 25-35 seconds prior to each wash cycle. Plate washing operations should be observed closely to confirm that all wells are being filled and aspirated completely.
- Set the wavelength of the spectrophotometer at 450 nm. For dual wavelength instruments, set a reference wavelength at 570 nm. If possible, set the microelisa plate reader or spectrophotometer to automatically subtract the absorbance reading of the substrate blank well from the absorbance reading of all other wells (see Figure 1, Recommended Plate Configuration).

Note: Some microelisa plate readers may require additional blanking of the instrument. Use a separate, uncoated strip for this purpose.

#### Setup Procedures

- See Figure 1 for the recommended configuration of the assay plate.

Figure1 Recommended Assay Configuration

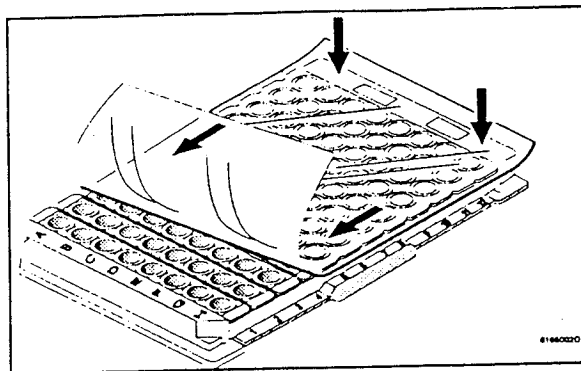


2. Identify the first well as the substrate blank well.
3. Identify negative control wells, positive control wells and test specimen wells.
4. Position the required number of microwell strips in the strip holder (8 wells per strip). If fewer than 12 strips are needed, use uncoated strips in the remaining positions when using a 96-well plate washer.
5. **Negative Controls**  
Prepare negative controls in triplicate on each 96-well plate. Deliver 200 $\mu$ L of undiluted Normal Human Serum (NHS) into three coated microtiter wells.
6. **Positive Controls**  
Prepare positive controls in duplicate on each 96-well plate. Deliver 200 $\mu$ L Normal Human Serum (NHS) into two coated microtiter wells, then add 50 $\mu$ L of the reconstituted HIV-1 p24 Antigen Reagent to each positive control well. Alternatively, deliver 250 $\mu$ L Diluted Positive Control to the positive control well.
7. **Test Specimens**  
Pipet 200 $\mu$ L of a test specimen to a designated coated well of the assay plate.

### Assay Procedures

1. **LYSE BUFFER**  
Add 20 $\mu$ L Lyse Buffer to all wells, except the substrate blank well. Prior to securing the plate sealer, ensure that the contents of each well are thoroughly mixed. Secure the plate by sealing along the edge with an adhesive plate cover as illustrated in Figure 2. Incubate the plate at 37°C +/- 2°C for 1 hour +/- 5 minutes.

Figure 2 Press all along the edges of the plate to securely seal the plate cover.



2. **WASH**  
Remove the cover from the plate and discard. Aspirate the solution from the wells. Add 300 $\mu$ L of Wash Buffer Working Dilution to each well. Allow wells to soak for 25-35 seconds. Aspirate the solution from the wells. Wash five (5) more times for a total of six washes. After the final wash step, grasp plate firmly along the edges, invert plate over absorbent paper and tap the plate gently to remove any remaining liquid.

**Important:** The time between the wash step and the addition of the next reagent must be less than five (5) minutes.

3. **Biotin Reagent**  
Add 200 $\mu$ L of reconstituted Biotin Reagent to all wells, except the substrate blank well. Cover the plate using a new plate cover and incubate at 37°C +/- 2°C for 1 hour +/- 5 minutes.
4. **WASH**  
Remove the cover from the plate and discard. Aspirate and wash six times as described in Step 2.
5. **SA-HRPO**  
Add 200 $\mu$ L of SA-HRPO Working Dilution to all wells, except the substrate blank well. Cover the plate and incubate for 30 +/- 2 minutes at 37°C +/- 2°C.
6. **WASH**  
Remove the plate cover and discard. Aspirate and wash six times as described in Step 2.
7. **TMB-SUBSTRATE**  
Add 200 $\mu$ L of TMB-Substrate Solution to ALL WELLS.  
Incubate for 30 +/- 2 minutes at room temperature (15-30°C).
8. **CSR-1**  
Add 50 $\mu$ L of CSR-1 to ALL WELLS.

**Important:** Add CSR-1 to the wells in the same sequence and at the same rate of speed that the TMB-Substrate Solution was added in step 7.

**Caution:** CSR-1 (4N H<sub>2</sub>SO<sub>4</sub>) contains 4N Sulfuric Acid solution (for which 2M and 4N are equivalent) and should be handled with adequate precautions.

9. **Absorbance at 450/570 nm**

Within 30 minutes of adding CSR-1 to the wells, determine the absorbance of all the wells at 450/570 nm.

### RESULTS

#### Calculation of Control Values and Cutoff Values

**Note:** Before proceeding with calculations, subtract the absorbance reading of the substrate blank well from all other absorbance values (if this was not subtracted automatically by the plate reader).

### Negative Control Values

1. Calculate the mean negative control value by adding the absorbance values for the three negative control wells and dividing by 3.

Example:	Sample No.	Absorbance
	1	0.058
	2	0.046
	3	<u>0.058</u>
		0.162

The mean negative control value is 0.162 divided by 3 = 0.054.

2. Each negative control well must give an absorbance value equal to or greater than 0.000 and equal to or less than 0.100.
3. If the absorbance of one of the three negative control values is greater than 0.100, that value should not be used to calculate the mean negative control value. Average the absorbance values for the remaining two negative control wells to determine the mean negative control value. If two or more negative control values are greater than 0.100, the test is invalid and should be repeated.

### Positive Control Values

1. Calculate the mean positive control value by adding the absorbance values for the two positive control wells and dividing by 2.

Example:	Sample No.	Absorbance
	1	1.000
	2	<u>1.215</u>
		2.215

The mean positive control value is 2.215 divided by 2 = 1.108.

2. The absorbance value of each positive control must be within 25% of the mean positive control. (For the example shown, the absorbance value for each positive control well should be between 0.942 and 1.274.) If the absorbance of either of the positive control values is outside of this range, the test is invalid and should be repeated.
3. The range for the expected absorbance value of the mean positive control is 0.60 to 1.60. A mean positive control that is less than 0.60 may indicate reagent deterioration or technical error, and the test is invalid and should be repeated.

### Cutoff Value

1. The cutoff value is the mean absorbance value for the three negative controls plus a constant factor of 0.055.

Example: The mean negative control is 0.054 + a constant factor 0.055 = 0.109. Therefore, the cutoff value is 0.109 for this example.

2. The expected range for the cutoff value is between 0.055 and 0.155. If the calculated cutoff value falls outside of this range, the test is invalid and should be repeated.

### Interpretation of Results

1. Specimens with an absorbance value that is less than the calculated cutoff value in the initial test are considered not reactive by the criteria of the COULTER HIV-1 p24 Antigen Assay and may be considered negative for HIV-1 p24 antigen. No further testing is required.
2. Specimens with absorbance values equal to or greater than the calculated cutoff value are considered initially reactive for HIV-1 p24 antigen by the criteria of the COULTER HIV-1 p24 Antigen Assay. Before interpretation, specimens that are initially reactive for HIV-1 p24 antigen should be retested in duplicate with the COULTER HIV-1 p24 Antigen Assay.
3. Initially reactive specimens which do not react in either of the duplicate repeat tests may be considered negative for HIV-1 p24 antigen. No further testing is required.
4. If the absorbance value of either or both of the duplicate tests is equal to or greater than the calculated cutoff value, the specimen is considered repeatedly reactive for HIV-1 p24 antigen by the criteria of the COULTER HIV-1 p24 Antigen Assay. Before interpretation, specimens that are repeatedly reactive should be tested using the additional, more specific COULTER HIV-1 p24 Antigen Neutralization Kit.
5. Specimens that are repeatedly reactive in the COULTER HIV-1 p24 Antigen Assay which are neutralized by the criteria of the COULTER HIV-1 p24 Antigen Neutralization Kit are considered positive for HIV-1 p24 antigen.
6. Specimens that are repeatedly reactive in the COULTER HIV-1 p24 Antigen Assay but do not have a valid neutralization test by the criteria of the COULTER HIV-1 p24 Antigen Neutralization Kit assay, i.e. specimens that are incubated with the Negative Neutralizing Control reagent that produce an absorbance less than the assay cutoff value, are considered indeterminate. Indeterminate results should be followed-up by repeat testing of the original specimen and testing a fresh specimen obtained at least 8 weeks later.
7. Specimens that are repeatedly reactive in the COULTER HIV-1 p24 Antigen Assay, but are negative, i.e. non-neutralizing, according to the criteria of the COULTER HIV-1 p24 Antigen Neutralization Kit, are considered negative for the neutralization test and indeterminate for HIV-1 p24 antigen. The interpretation of results of specimens found to be repeatedly reactive in the COULTER HIV-1 p24 Antigen Assay and negative in the additional, more specific COULTER HIV-1 p24 Antigen Neutralization assay, is unclear. The majority of such specimens do not contain HIV-1 p24 antigen, however, false negative neutralization tests can occur with some specimens that do contain HIV-1 p24 antigen. Further clarification may be obtained by retesting the original specimen or testing a fresh specimen for HIV-1 p24 antigen. Reactivity associated with seroconversion and the HIV-1 "window period" may be resolved by obtaining a fresh specimen after 8 weeks and testing for HIV-1 antibody.

## COULTER PROCEDURE FOR QUANTITATION OF HIV-1 P24 ANTIGEN IN BLOOD AND TISSUE CULTURE SUPERNATANTS

### REAGENT PREPARATION AND STORAGE

Preparation of Calibration Standards for Quantitation of p24 Antigen:

1. Prepare five Calibration Standards by serially diluting reconstituted HIV-1 p24 Antigen Reagent with diluent as shown below. When serum or plasma specimens are being assayed, the diluent should consist of Normal Human Serum (NHS) provided in the kit. NHS is provided in the kit in sufficient volume to perform two calibration curves in duplicate. When tissue culture supernatants are being assayed, the diluent should consist of supernatant from uninfected cell cultures or tissue culture media containing the same supplements as the test specimens.

Tube	Diluent	+	Antigen Reagent	Final p24 Antigen Concentration (pg/mL)
A	0.8 ml	+	0.2 ml Reconstituted	125.0
B	0.5 ml	+	0.5 ml from A	62.5
C	0.5 ml	+	0.5 ml from B	31.3
D	0.5 ml	+	0.5 ml from C	15.6
E	0.5 ml	+	0.5 ml from D	7.8

**Note:** Each well for testing a Calibration Standard requires 200 $\mu$ L of diluted material (tubes A through E).

The Calibration Standards should be assayed in duplicate.

### SPECIMEN COLLECTION, PREPARATION AND STORAGE

1. Plasma collected in acid-citrate-dextrose (ACD), citrate-phosphate-dextrose with adenine (CPDA-1), EDTA, sodium citrate or heparin, or serum may be used and should be tested as soon as possible following collection. No special preparation of the patient is required prior to blood collection.
2. Because of the instability of the analyte, all samples should be tested for HIV-1 antigen as soon as possible after they are drawn, or else promptly stored at -20°C pending testing. Where operational conditions preclude rapid testing or frozen storage, signal loss can be minimized by limiting pre-test storage at room temperature (not exceeding 26°C) and refrigeration (nominal 4°C) to a maximum of 7 days, including no more than three days at room temperature. If the initial EIA, the repeat EIA, and the neutralization test cannot be completed within 3 days, specimens may be stored frozen at -20°C +/- 2°C or -70°C +/- 5°C for up to 3 years. Use of self-defrosting freezers is not recommended. The effect of longer storage on the stability and detectability of HIV-1 p24 antigen is unknown.
3. Remove the serum from the clot or plasma from the red cells as soon as possible to avoid hemolysis. COULTER HIV-1 p24 Antigen Assay and COULTER HIV-1 p24 Neutralization assay are not affected by elevated hemoglobin up to 350 mg/dL or triglycerides up to 474 mg/dL. —
4. Specimens containing particulate matter may give inconsistent test results. Such specimens should be clarified by centrifugation prior to assay.
5. Avoid subjecting specimens to repeated freeze/thaw cycles. Studies show that accurate test results can be obtained for specimens subjected to five freeze/thaw cycle. More than five freeze/thaw cycles may cause inaccurate results.
6. Plasma and serum specimens should be initially used without dilution. Use Normal Human Serum (NHS) reagent to prepare dilutions of plasma and serum specimens, if necessary.
7. Tissue culture supernatants may require dilution.  
Supernatant from uninfected cell cultures or tissue culture media containing the same supplements as the test specimens should be used for negative and positive controls and as the diluent for preparation of the Calibration Standards.
8. For purposes of quantitating the amount of p24 antigen in a specimen, if the absorbance for an undiluted specimen exceeds the range of the Calibration Standards, prepare dilutions of the specimen and test again.
9. Bring all specimens to room temperature (15-30°C) prior to assay.

#### Quantitation Assay Setup Procedures

1. See Figure 1, for the recommended configuration of the positive and negative controls.
2. Identify the first well as the substrate blank well.
3. Identify negative control wells, positive control wells, Calibration Standard wells and test specimen wells.
4. Position the required number of microwell strips in the strip holder (8 wells per strip). If fewer than 12 strips are needed, use uncoated strips in the remaining positions when using a 96-well plate washer.
5. Negative Controls  
Prepare negative controls in triplicate on each 96-well plate.  
  
Serum or plasma: Deliver 200 $\mu$ L of undiluted Normal Human Serum (NHS) into three coated microtiter wells.  
  
Tissue Culture Supernatant: Deliver 200 $\mu$ L of supernatant from uninfected cell cultures or tissue culture media containing the same supplements as the test specimens into three coated microtiter wells.
6. Positive Controls  
Prepare positive controls in duplicate on each 96-well plate.

Serum or plasma: Deliver 200 $\mu$ L Normal Human Serum (NHS) into two coated microtiter wells, then add 50 $\mu$ L of the reconstituted HIV-1 p24 Antigen Reagent to each positive control well.

Tissue Culture Supernatant: Deliver 200 $\mu$ L of supernatant from uninfected cell cultures or tissue culture media containing the same supplements as the test specimens into two coated microtiter wells, then add 50 $\mu$ L of the reconstituted HIV-1 p24 Antigen Reagent to each positive control well.

7. **Calibration Standards**

In addition to the negative and positive control wells described above, prepare one negative Calibration control in duplicate on each 96-well plate. Deliver 200 $\mu$ L of the same diluent used to prepare Calibration Standards into two coated microtiter wells.

Prepare five Calibration Standards in duplicate on each 96-well plate, or partial plate, used. Deliver 200 $\mu$ L of each Calibration Standard into two coated microtiter wells.

8. **Test Specimens**

Pipet 200 $\mu$ L of a test specimen to a designated coated well of the assay plate.

**Assay Procedures**

Follow assay procedures described for the qualitative assay.

**QUANTITATIVE ASSAY RESULTS**

Calculations of Negative and Positive Control Values and Cutoff Value

Follow the calculations and criteria described for the qualitative assay.

**Note:** Tissue culture supernatants or tissue culture media may produce absorbances greater than that expected for serum.

**Quantitation of p24 Antigen Using Calibration Standards**

1. The negative control values, positive control values and cutoff value must meet the recommended criteria or the test is invalid and should be repeated.

2. Plot the absorbance value for each Calibration Standard and draw a best-fit Calibration Curve manually, or perform linear regression analysis with appropriate computer and software as illustrated in Figure 5 of the Performance Characteristics section of this package insert. The expected ranges of absorbance values for Calibration Standards are presented in Table 13, and the expected range of the slope and the correlation coefficient ( $r$ ) for a Calibration Curve is presented in the text accompanying Table 13.

3. Determine the concentration of HIV-1 p24 antigen in a test specimen by comparing the absorbance of the test specimen with a corresponding absorbance estimated from the Calibration Curve manually or using appropriate statistical software.

**Note:** The absorbance value ( $OD_{450\pm 70\text{ nm}}$ ) for a test specimen must lie within the range of the calibration curve to accurately determine the concentration of HIV-1 p24 antigen in the specimen.

4. Specimens with absorbance values higher than the highest point on the calibration curve, 125  $\mu$ g/mL, may be serially diluted and tested. The absorbance values for the serial dilutions (three to five points) should be linear. Determine the concentration of HIV-1 p24 antigen in a diluted specimen by comparing the absorbance with a corresponding absorbance estimated from the Calibration Curve.

**Limitation of Quantitation:**

Quantitation of HIV-1 p24 antigen in some diluted clinical specimens may not be possible due to the complicated and unpredictable dissociation of antigen and antibody complexes in diluted specimens. In these cases, absorbance values for serial dilutions of the clinical specimens will not be linear.

**Note:** Quantitation of the HIV-1 p24 antigen level in a clinical specimen is not required for test results to aid in the diagnosis and prognosis of HIV-1 infection.

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**LIMITATIONS OF THE PROCEDURE**

Data obtained from testing blood specimens of known HIV-1 infected persons and persons at low risk for HIV-1 infection suggest that repeatedly reactive specimens with high absorbance values are more likely to demonstrate the presence of HIV-1 p24 antigen by additional testing (the Neutralization Kit assay). If the absorbance values for the initial test, the repeat test and the neutralization test are near the calculated cutoff value, a fresh specimen should be obtained from the individual at least 8 weeks later and tested.

AIDS and conditions related to AIDS are clinical syndromes, and their diagnosis can only be established clinically. HIV-1 p24 antigen testing alone cannot be used to diagnose AIDS, even after the presence of p24 antigen is confirmed by additional testing. A person who has HIV-1 p24 antigen is presumed to be infected with the AIDS virus; appropriate counseling, antibody testing and medical follow-up should be offered. Medical diagnosis and evaluation should include confirmation of all test results using a freshly drawn specimen. Clinical studies suggest that testing specimens in the COULTER HIV-1 p24 Antigen Assay and the COULTER HIV-1 p24 Antigen Neutralization Kit assay may aid in the clinical evaluation of disease progression.

False negative results may occur because free antigen levels are below the lower limit of detection of this assay. This may occur during the earliest stage of infection before antigens reach detectable levels.

The presence of antigen-antibody complexes in clinical specimens may obscure detection of HIV-1 p24 antigen. Antigen levels in some clinical specimens may decline over time due to degradation, or due to improper handling, storage or testing. Although there is wide variation in the stability of antigens in individual clinical specimens, evidence of antigen instability occurs more frequently for clinical specimens with low levels of p24 antigen (see Performance Characteristics section of this package insert for more information).

The possibility of previous exposure to or infection with HIV-1 cannot be excluded by a negative HIV-1 antigen test result.

False positive results may occur due to non-specific binding to assay materials, and not from infection with HIV-1. It is recommended that a fresh specimen be obtained from the individual for testing and evaluation.

The COULTER HIV-1 p24 Antigen Assay procedure and the Interpretation of Results must be followed closely when testing for the presence of HIV-1 p24 antigen in individual specimens. The assay procedures must be followed closely because the procedures were designed to test individual specimens of serum, plasma and tissue culture supernatants. Insufficient data are available to interpret test results performed on other body fluid specimens, pooled blood, processed plasma or

products made from such pools. Testing of these specimens is not recommended and is not considered predictive of test results for specimens collected at a later time from the same person.

Quantitation of p24 antigen in some diluted clinical specimens may not be possible due to formation and dissociation of antigen-antibody complexes upon specimen dilution. In these cases, absorbance values with serial dilutions of the clinical specimens will not be linear.

## PERFORMANCE CHARACTERISTICS OF THE COULTER HIV-1 p24 ANTIGEN ASSAY

### I. PRECISION AND REPRODUCIBILITY

#### A. Spiked Specimens

The precision with which HIV-1 p24 antigen is detected by the COULTER HIV-1 p24 Antigen Assay was evaluated at four different test sites using a Proficiency Panel of three serum specimens spiked with a known amount of purified HIV-1 p24 antigen. The intra-assay and inter-assay precision data from testing these specimens are presented in Table 1.

Table 1. Test Precision Using Proficiency Panel Specimens

Panel Member	Antigen Spike (pg/mL)	Site	(n)	Mean OD	Qualitative Assay						Quantitative Assay					
					Intra-Assay			Inter-Assay			Intra-Assay			Inter-Assay		
					Mean S/CO <sup>2</sup>	SD	%CV	S/CO	SD	%CV	Mean (pg/mL)	SD	%CV	Mean	SD	%CV
1	34.3	A	(5)	0.232	2.8	0.14	5.0	3.2	0.97	30.3	32.2	1.9	5.9	34.4	3.5	10.2
		B	(10)	0.285	4.6	0.37	8.0				37.0	3.8	10.2			
		C	(10)	0.308	2.9	0.26	9.0				36.9	2.2	6.0			
		D	(5)	0.203	2.4	0.12	5.0				31.0	1.8	5.8			
2	56.6	A,B,C,D									58.0	2.1	3.6			
		A	(5)	0.384	4.6	0.15	3.3	58.0	2.1	3.6						
		B	(10)	0.486	7.8	0.75	9.6	61.8	3.6	5.8						
		C	(5)	0.358	4.2	0.27	6.4	58.4	4.1	7.0						
3	112	A,B,C,D					5.3	1.68	31.7	104.6	13.5	12.9	59.7	3.6	6.0	
		A	(5)	0.659	7.9	0.95	12.0	104.6	13.5	12.9						
		B	(10)	0.888	14.3	1.44	10.10	111.9	5.7	5.1						
		C	(5)	0.65	7.6	0.26	3.4	110.1	3.9	3.5						
A,B,C,D										111.1	7.2	6.5	109.9	7.8	7.1	
	D	(5)	0.93	9.4	0.58	6.2	9.8	3.10	31.6	111.1	7.2	6.5	109.9	7.8	7.1	

\* S/CO = signal to cutoff. A ratio of 1.00 or greater is considered reactive for HIV-1 p24 Antigen

#### B. Fresh and Frozen Clinical Specimens

The reproducibility of the assay for HIV-1 p24 antigen was examined at four clinical sites using paired fresh and frozen serum or plasma specimens. Fresh specimens were obtained from blood donors and individuals who were previously identified as reactive for HIV-1 antibody and HIV-1 antigen using FDA-licensed tests. Portions of each clinical specimen were frozen. Coded panels of fresh (2-8°C) and frozen (-20°C) specimens were prepared at COULTER and distributed for testing using multiple kit lots on three days. Test results were independent of the fresh or frozen condition of the clinical specimen, study site, test day, technician and kit lot. Based on 96 determinations, Table 2 shows the mean absorbance value, the mean concentration of HIV-1 p24 antigen and Signal/cutoff with the respective standard deviation (SD) and the percent coefficient of variation (%CV) for each panel member.

Table 2. Reproducibility of the Assay for HIV-1 P24 Antigen Using Paired Fresh and Frozen Clinical Specimens

Panel Member	Absorbance Mean OD	Signal/CO	Mean pg/mL	SD	%CV
1	0.682	8.86	66.7	8.97	13.4
2	0.551	7.16	53.7	7.05	13.1
3	0.844	10.96	84.4	7.65	9.1
4	0.530	6.88	51.1	7.36	14.4
5	0.602	7.82	59.3	6.46	10.9
6	0.190	2.47	16.3	2.87	17.6
7	0.009				below cutoff*
8	0.022				below cutoff

\* S/CO = signal to cutoff. A ratio of 1.00 or greater is considered reactive for HIV-1 p24 Antigen

### II. SPECIFICITY

#### A. Specificity of the COULTER HIV-1 p24 Antigen Assay in Blood Donors

Serum or plasma specimens from 301,699 normal blood donors were screened for HIV-1 p24 antigen using the COULTER HIV-1 p24 Antigen Assay at eight blood banks. The study was conducted by the American Association of Blood Banks (AABB) as part of a larger study to examine the seroprevalence of HIV-1 antigen in low risk subjects. Results of testing are presented in Table 3.

Table 3. HIV-1 Antigen Seroprevalence Study Performed by the AABB Centers

AABB Study Site	Number of Subjects	Number Initially Reactive (percent)	Number Repeatedly Reactive (percent)	Number Neutralized <sup>a</sup>
1	60,644	111 (0.18)	11 (0.018)	0
2	23,655	183 (0.78)	9 (0.038)	0
3	30,042	173 (0.59)	24 (0.080)	0
4	61,571	352 (0.57)	30 (0.044)	0
5	60,043	320 (0.53)	36 (0.060)	0
6	22,564	40 (0.18)	9 (0.040)	0
7	16,905	35 (0.21)	4 (0.024)	0
8	26,275	131 (0.50)	20 (0.076)	2 <sup>c</sup>
Total	301,699	1,345 (0.45)	143 (0.047)	2 <sup>c</sup>

- a The American Association of Blood Banks (AABB) performed this study under investigational new drug (IND) protocol. COULTER Corporation supplied the test kits to the study sites.
- b Specimens found to be repeatedly reactive at the blood bank sites were tested again at an AABB central laboratory. Thirty-three specimens were found to be repeatedly reactive at the central laboratory and were tested further using the COULTER HIV-1 p24 Antigen Neutralization Kit.
- c Specimens were positive for HIV-1 antibody by Western blot analysis.

Among the 301,699 specimens from normal blood donors tested using the COULTER HIV-1 p24 Antigen Assay, 1,345 (0.45%) were initially reactive and 143 (0.047%) were repeatedly reactive. Thirty-three specimens were found to be repeatedly reactive at a central AABB testing site. Two repeatedly reactive specimens were found to contain HIV-1 p24 antigen using the additional, more specific COULTER HIV-1 p24 Antigen Neutralization Kit, and were also found to contain antibodies to HIV-1 using Western blot analysis. Additional tests of specimens that were repeatedly reactive in the COULTER HIV-1 p24 Antigen Assay and negative for p24 antigen in the Neutralization test using Western blot or DNA PCR were all negative.<sup>25</sup>

The specificity of the COULTER HIV-1 p24 Antigen Assay based on an assumed zero prevalence of HIV-1 p24 antigens in random blood donors who have no other evidence of HIV-1 infection, is estimated to be 99.95% (301,556/301,697) based on additional testing using the COULTER HIV-1 p24 Antigen Neutralization Kit and Western blot analysis for HIV-1 antibody.

**B. Specificity of the COULTER HIV-1 p24 Antigen Assay in Patients with Potentially Interfering Medical Conditions**

The specificity of the COULTER HIV-1 p24 Antigen Assay was investigated by testing serum or plasma specimens from 104 patients with various medical conditions, including viral infections, bacterial infections, parasitic diseases and autoimmune disorders. Test results are shown in Table 4. None of these specimens from those patients were reactive.

Table 4. Reactivity of the COULTER HIV-1 p24 Antigen Assay for Sera from Individuals with Potentially Interfering Medical Conditions

Disease Condition	Number Tested	Number Reactive
Rheumatoid Factor	14	0
Chlamydia	8	0
Legionnaires	2	0
Toxoplasmosis	8	0
Herpes simplex virus	12	0
HIV-2	6	0
HTLV-III	25	0
Epstein-Barr virus	12	0
Cytomegalovirus	14	0
Carcinoma	2	0
Mycoplasma	1	0
Total	104	0

**III. SENSITIVITY**

**A. Prevalence of HIV-1 p24 Antigen in Clinical Groups**

The performance characteristics of the COULTER HIV-1 p24 Antigen Assay and the COULTER HIV-1 p24 Antigen Neutralization Kit were studied with serum or plasma specimens obtained retrospectively from 585 HIV-1 antibody positive patients. Over a two to three year period, an average of 4.1 specimens were collected from each of these 585 individuals classified into AIDS (CDC IV), AIDS-related complex (ARC, CDC III) or asymptomatic (CDC II) groups.<sup>26,27</sup> Patient specimens were tested for the presence of HIV-1 p24 antigen in a blinded manner at four clinical study sites. In addition, performance was evaluated in in-house studies using serum or plasma specimens of 173 blood donors who were HIV-1 antibody positive. Specimens were considered positive for HIV-1 p24 antigen when test results were repeatedly reactive in the HIV-1 p24 Antigen Assay and positive in the HIV-1 p24 Antigen Neutralization assay. Test results using the first specimen collected from each study subject are presented in Table 5, as well as the prevalence of measurable HIV-1 p24 antigen in various clinical groups.

Table 5. Prevalence of HIV-1 p24 Antigen in Clinical Groups

Clinical Group (CDC Class)	Test Site	Number of Subjects Tested	Number Initially Reactive IR (%)	Number Repeatedly Reactive RR (%)	Number of RRs with a Valid Neut Test <sup>a</sup>	Number Neutralized (%)	Prevalence of HIV-1 p24 Antigen (%)
AIDS (CDC IV)	Field	211	110(52.1)	99(46.9)	90	90 (100)	42.7
ARC (CDC III)	Field	187	43(23.0)	41(21.9)	37	37 (100)	19.8
Asymptomatic (CDC II)	Field	187	35(18.7)	31(16.6)	22	21 <sup>c</sup> (95.5)	11.2
Asymptomatic (CDC II)	Coulter <sup>d</sup>	173	14( 8.1)	14( 8.1)	14	14 (100)	8.1
Total		758	202(26.6)	185(24.4)	163	162 (99.4)	21.4

Abbreviations: IR, initially reactive; RR, repeatedly reactive; Neut, Neutralization

- Clinical Groups are based on CDC Classification of HIV Disease, published in MMWR 1986; 35: 334-339.
- Among the 185 specimens that were repeatedly reactive in the HIV-1 p24 Antigen Assay, 22 specimens did not produce a valid signal with the Negative Neutralizing Control (NNC). All 22 specimens had low levels of p24 antigen, ranging from 5.4 pg/ml to 27 pg/mL and were stored at 2-8°C more than 3 days prior to testing in the HIV-1 p24 Antigen Neutralization assay. In-house studies of storage conditions showed that the level of p24 antigen, and the signal produced with the NNC, declined for some clinical specimens stored at 2-8°C. This effect was attributed to instability of HIV-1 p24 antigen when specimens are stored at 2-8°C, since paired specimens stored frozen at -20°C or -70°C did not exhibit a decline.
- One specimen with a valid neutralization test did not neutralize. This specimen had a low level of p24 antigen (13.9 pg/mL in the initial assay), NNC signal to cutoff 1.2, and was stored at 2-8°C for 10 days prior to testing in the neutralization assay.
- Testing was performed within five days.

Results show that of the 758 specimens tested by the COULTER HIV-1 p24 Antigen Assay from patients known to be HIV-1 antibody positive, 202(26.6) were initially reactive, 185 (24.4%) were repeatedly reactive and 162 (21.4%) were positive in the supplemental HIV-1 p24 Antigen Neutralization test.

Among the 185 specimens that were repeatedly reactive in the HIV-1 p24 Antigen Assay, 22 specimens did not produce a valid signal with the Negative Neutralizing Control (NNC), i.e. the absorbance value was below the calculated cutoff. In the initial screening test, all 22 specimens had low levels of HIV-1 p24 antigen ranging from 5.4 pg/ml to 27 pg/mL and were stored at 2-8°C for more than 3 days prior to testing in the HIV-1 p24 Antigen Neutralization assay. In-house studies of storage conditions showed that the p24 antigen levels and the signal produced with the NNC declined for some specimens stored at 2-8°C. Paired specimens stored frozen at -20°C did not exhibit a decline in the level of p24 antigen or a decline in the signal with the NNC with time. The observed decrease in measurable HIV-1 p24 antigen was attributed to the instability of HIV-1 p24 antigen when specimens are stored at 2-8°C.

Among the 163 clinical trial specimens that produced a valid neutralization test signal with the NNC, 162 (99.4%) were neutralized. The one specimen that did not neutralize was stored at 2-8°C for 10 days prior to testing in the neutralization assay.

Based on the positive test results in the COULTER HIV-1 p24 Antigen Assay and the COULTER HIV-1 p24 Neutralization test, the prevalence of HIV-1 p24 antigen was estimated to be 42.7% for patients with AIDS, 19.8% for patients with ARC and 9.7% for asymptomatic patients, which is comparable to previous estimates for HIV-1 antigens in these clinical groups using HIV-1 antigen tests without treatment to dissociate antigen-antibody complexes.

#### B. Performance on Seroconversion Panels

The ability of the COULTER HIV-1 p24 Antigen Assay to detect HIV-1 p24 antigen was evaluated using seroconversion panels obtained from a commercial source (Boston Biomedica, Inc., West Bridgewater, MA). The panels consisted of well characterized plasma specimens that were sequentially obtained from blood donors who seroconverted over the course of their donation history. Qualitative test results are presented in Table 6. Results show that the COULTER HIV-1 p24 Antigen Assay detected HIV-1 p24 antigen prior to the appearance of detectable HIV-1 antibodies by approximately 21 days in Panel A, 23 days in Panel C, 42 days in Panel E and 28 days in Panel G. HIV-1 p24 antigen was not detected in Panel D, in which one specimen was positive for HIV-1 antibody.

Table 6. HIV-1 p24 Antigen Detection in Seroconversion Panels

Panel	Panel Member	Day of Donation	Antibody Status <sup>a</sup>	p24 Antigen Assay OD	p24 Antigen S/CO <sup>b</sup>	Antigen Status
A	BBI-1	1	-	0.024	0.24	-
	2	65	-	0.053	0.54	-
	3	87	-	1.757	17.93	+
	4	108	+	0.060	0.61	-
	5	122	+	0.066	0.67	-
	6	129	+	0.047	0.48	-
	7	136	+	0.045	0.46	-
	8	143	+	0.045	0.46	-
	9	164	+	0.093	0.95	-
C	BBI-20	1	-	0.176	2.29	+
	21	8	-	1.994	25.90	+
	22	10	-	1.801	23.39	+
	23	15	-	0.067	0.87	-
	24	17	-	0.047	0.61	-
	25	22	-	0.044	0.57	-
	26	24	+	0.031	0.40	-
	27	29	+	0.034	0.44	-
	28	31	+	0.032	0.42	-
	29	36	+	0.042	0.55	-
	30	43	+	0.038	0.49	-
	31	45	+	0.038	0.49	-
	32	57	+	0.026	0.34	-
	33	59	+	0.026	0.34	-
	34	64	+	0.031	0.40	-
	35	66	+	0.055	0.71	-
	36	71	+	0.046	0.60	-
37	73	+	0.041	0.53	-	
D	BBI-40	1	-	0.015	0.21	-
	41	22	-	0.039	0.56	-
	42	50	-	0.037	0.53	-
	43	92	-	0.027	0.39	-
	44	99	+	0.027	0.39	-
E	BBI-50	1	-	0.044	0.63	-
	51	8	-	0.023	0.33	-
	52	22	-	0.025	0.36	-
	53	36	-	0.027	0.39	-
	54	43	-	0.027	0.39	-
	55	50	-	0.018	0.26	-
	56	64	-	0.030	0.43	-
	57	85	-	0.548	7.83	+
	58	92	-	1.990	28.43	+
59	127	+	0.050	0.71	-	
G	BBI-80	1	-	1.953	30.05	+
	81	4	-	1.953	30.05	+
	82	8	-	1.953	30.05	+
	83	12	-	1.999	30.75	+
	84	15	-	0.592	9.11	+
	85	19	-	0.078	1.20	+
	86	25	-	0.018	0.28	-
	87	29	+	0.015	0.23	-
	88	33	+	0.088	1.35	+
	89	36	+	0.077	1.18	+
	90	154	+	0.025	0.38	-
91	168	+	0.017	0.26	-	

Abbreviations: BBI, Boston Biomedica, Inc.; S/CO, signal to cutoff ratio.

a Abbott HIVAB HIV-1 antibody EIA test.

b A S/CO ratio of 1.00 or greater is considered reactive for HIV-1 p24 antigen.

C. **Additional Studies Using Seroconversion Panels**

The performance of the COULTER HIV-1 p24 Antigen Assay for specimens obtained from subjects immediately prior to seroconversion, that is, during the "HIV-1 window period," was evaluated by independent investigators using 31 seroconversion panels. The time of seroconversion was based on antibody testing using licensed antibody screening tests (Abbott Laboratories HIVAB HIV-1/HIV-2 (rDNA) EIA and Genetic Systems™ HIV-1/HIV-2 EIA). Panel specimens were also tested using the licensed polyclonal HIV-1 antigen assay and two investigational monoclonal HIV-1 p24 antigen tests, as well as two investigational HIV-1 RNA tests. Test results indicate that the COULTER HIV-1 p24 Antigen Assay detected HIV-1 p24 antigen prior to seroconversion in 25 (80.6%) of the 31 seroconversion panels tested and coincident with seroconversion in 3 panels (9.7%). For two panels, none of the antigen tests detected antigen while only one of the RNA tests detected HIV-1 RNA, and for another panel, neither antigen nor RNA were detected. Among the 28 seroconversion panels with evidence of antigen, the COULTER test detected HIV-1 p24 antigen earlier than the licensed polyclonal antigen test in 6 of the panels (21.4%) and earlier than other monoclonal p24 antigen tests in 5 (17.9%) and 3 (10.7%) of the panels. In addition, the COULTER test detected HIV-1 p24 antigen coincident with the detection of HIV-1 RNA (both tests) in all but one panel in which the COULTER test detected HIV-1 p24 antigen two days later.

D. **Studies of the Monoclonal HIV-1 p24 Antigen Assay and a Polyclonal Test for HIV-1 Antigens Using Specimens from Antibody Positive Patients**

A polyclonal capture assay intended to detect multiple HIV-1 antigens, including p24 antigen, was previously licensed by the FDA as an aid in the diagnosis and prognosis of HIV infection, and was not approved for blood donor screening. The relative sensitivity of the COULTER monoclonal test for HIV-1 p24 antigen and the polyclonal test for HIV-1 multiple antigens were evaluated using antibody positive specimens prescreened for the presence of HIV-1 antigen by either the monoclonal or polyclonal test and antibody positive specimens of unknown antigen reactivity.

**Specimens Pre-screened by COULTER HIV-1 p24 Antigen Assay**

A study was conducted using 207 serum or plasma specimens obtained from HIV-1 antibody positive patients that tested positive for HIV-1 p24 antigen using the COULTER HIV-1 p24 Antigen Assay system, i.e. repeatedly reactive in the screening assay and positive in the neutralization assay. Within 24 hours, p24 antigen positive specimens were tested using a previously licensed polyclonal assay for HIV-1 antigens. Results are presented in Table 7.

**Table 7. p24 Antigen Detection in Specimens Pre-screened Using the COULTER Assay**

HIV-1 Antigen Assay	Initial Assay	
	Not Reactive	Reactive
COULTER HIV-1 p24 Antigen Assay	0	207
Previously Licensed Assay	36	171

The previously licensed polyclonal assay detected HIV-1 antigens on the initial test in 82.6% (171/207) of the specimens in which p24 antigen was detected using the COULTER HIV-1 p24 Antigen Assay and COULTER HIV-1 p24 Antigen Neutralization assay, and failed to detect HIV-1 antigens in 17.4% (36/207) of the specimens.

**Studies Using Antigen Positive Specimens Pre-screened by a previously licensed polyclonal assay**

A retrospective study of 396 serum or plasma specimens shown to be repeatedly reactive for HIV-1 antigens using a previously licensed polyclonal assay was conducted by an independent investigator. The study population included 44 patients of unknown disease classification, 64 CDC II patients, 154 CDC III patients, and 134 CDC IV patients. Specimens were tested using the COULTER HIV-1 p24 antigen assay. Complete test results are included in Table 8.

**Table 8. P24 Antigen Detection in Specimens Pre-screened Using a Previously Licensed Polyclonal Assay**

HIV-1 Antigen Assay	Initial Assay		Repeat Assay		Neutralization Assay		
	Not Reactive	Reactive	Not Reactive	Reactive	Negative	Indeterminate	Positive
Previously Licensed Assay	0	396	0	396	Neutralization Not Done		
COULTER HIV-1 p24 Antigen Assay	48	348	8 <sup>a</sup>	340	3 <sup>b</sup>	4 <sup>c</sup>	333

- a. Specimens had p24 antigen levels < 7.8 pg/mL (OD < 0.10) and were held at 2-8°C for 9 days or more prior to repeat testing. All specimens were repeatedly reactive for p24 antigen and neutralization test positive after ICD (immune complex dissociation) pre-treatment to dissociate antigen-antibody complexes.
- b. Specimens had p24 antigen levels of 8.7, 19.7 and 87.4 pg/mL that gave OD values of 0.067, 0.219 and 0.721, respectively in the screening assay. Specimens were held at 2-8°C for 5 days or more prior to neutralization testing. All 3 specimens were p24 antigen positive in the neutralization test after ICD pre-treatment to dissociate antigen antibody complexes.
- c. Specimens were held at 2-8°C for 37 or more days prior to neutralization testing. All 4 specimens were p24 antigen positive in the neutralization test after ICD pre-treatment to dissociate antigen-antibody complexes.

The COULTER HIV-1 p24 Antigen Assay detected p24 antigen on the initial test in 87.9% (348/396) of the specimens shown to contain HIV-1 antigen using the previously licensed polyclonal assay, and failed to detect HIV-1 p24 antigen in 12.1% of the specimens. The possibility that detection of HIV-1 p24 antigen in these specimens was affected by the presence of p24 antigen-antibody complexes was investigated by pre-treating 46 of the 48 specimens with COULTER's investigational ICD (Immune Complex Dissociation) reagent to dissociate antigen-antibody complexes prior to testing in the HIV-1 p24 antigen assay. All 46 specimens treated with ICD reagent were repeatedly reactive in the COULTER HIV-1 p24 Antigen Assay and positive in the Neutralization assay, suggesting that p24 antigen-antibody complexes interfered with the detection of p24 antigen in these specimens.

**Antibody Positive Specimens Not Pre-screened for HIV-1 p24 Antigen**

The performance of the COULTER HIV-1 p24 Antigen Assay and COULTER HIV-1 p24 Antigen Neutralization test and that of a previously licensed HIV-1 antigen assay were evaluated using serum or plasma specimens obtained from 261 patients known to be positive for HIV-1 antibody. The study population included 140 CDC IV patients, 23 CDC III patients, 81 CDC II patients and 17 patients of unknown CDC classification. The assays differ in that the COULTER assay utilizes a p24-specific monoclonal antibody as the capture reagent and the previously licensed assay utilizes HIV-1-specific polyclonal antibodies as the capture reagent. Results of testing the clinical specimens are presented in Table 9.

Table 5. HIV-1 p24 Antigen Detection Using COULTER HIV-1 p24 Antigen Assay System and a Previously Licensed Polyclonal Assay System

HIV-1 Antigen Assay	Initial Assay		Repeat Assay		Neutralization Assay	
	Not Reactive	Reactive	Not Reactive	Reactive	Negative	Positive
COULTER HIV-1 p24 Antigen Assay and Previously Licensed Antigen Assay	156	79	2	77	0	77
Previously Licensed Antigen Assay Only	11	15	0	15	0	15
COULTER HIV-1 p24 Antigen Assay Only	15	11	6	5	0	5

The COULTER monoclonal assay system detected HIV-1 p24 antigen in 84% (77/92) of the specimens that were identified by the previously licensed polyclonal assay system as containing HIV-1 antigens. The COULTER assay system also detected HIV-1 p24 antigen in 5 specimens that were not identified by the polyclonal capture assay.

The presence of p24-specific antibodies in clinical specimens has been shown to interfere with the detection of p24 antigen. The fifteen specimens in which HIV-1 antigens were detected only by the polyclonal capture assay were examined for the presence of p24 antigen-specific antibodies. Using an investigational competitive immunoassay, tests showed that thirteen of the fifteen specimens contained antibodies that effectively competed for binding to HIV-1 p24 antigen. It is likely that the presence of p24-specific antibodies in these specimens accounts for the fact that no p24 antigen was detected using the p24-specific monoclonal capture assay which was performed without taking steps to first dissociate antigen-antibody complexes.

Based on an assumed 100% prevalence of HIV-1 p24 antigen in specimens obtained from individuals who are positive for HIV-1 antibodies, the sensitivity of the COULTER HIV-1 p24 Antigen Assay was 84.5% (82/97) for this set of specimens compared with a sensitivity of 94.8% (92/97) for the previously licensed polyclonal assay.

#### IV. HIV-1 P24 ANTIGEN AS A PROGNOSTIC MARKER IN CLINICAL GROUPS

Disease progression of subjects who tested positive for HIV-1 p24 antigen was compared with that of subjects who tested negative for HIV-1 p24 antigen to assess HIV-1 p24 antigen as a prognostic marker. Disease progression was defined as reclassification to a more severe disease state. Kaplan-Meier curves were constructed for asymptomatic and ARC subjects identified in Table 5 when multiple specimens and relevant information about the subject's disease classification were available over a minimum of three months<sup>28</sup>. The analysis included 159 asymptomatic (CDC II) subjects and 141 ARC (CDC III) subjects, and excluded the 211 subjects whose disease had already progressed to AIDS. Baseline was defined as the time of the first specimen with known clinical status for subjects who were negative for HIV-1 p24 antigen and the time of the first positive specimen for subjects who were HIV-1 p24 antigen positive. The statistical analysis was limited to a 500 day period because 500 days was considered a clinically useful prognostic time frame.

##### A. Asymptomatic subjects (CDC II)

Figure 3 Panel A shows the Kaplan-Meier curves for asymptomatic subjects after they tested positive for HIV-1 p24 antigen or, for those who always tested negative, the initial test. The rate of disease progression for the 38 subjects who tested positive for HIV-1 p24 antigen was significantly faster than the rate of disease progression for the 121 subjects who tested negative for HIV-1 p24 antigen (p values were 0.02 by Wilcoxon and 0.04 by log rank statistical tests). For the HIV-1 p24 antigen positive subjects, there was a steady decline in the symptom-free survival to about 63% during the next 500 days, while for HIV-1 p24 antigen negative subjects there was a decline to about 83%. The odds ratio at day 500 for progression to disease is 2.9 for HIV-1 p24 antigen positive subjects relative to HIV-1 p24 antigen negative subjects. These results suggest that the COULTER HIV-1 p24 Antigen Assay may be useful as a prognostic indicator or in monitoring disease progression of asymptomatic subjects.

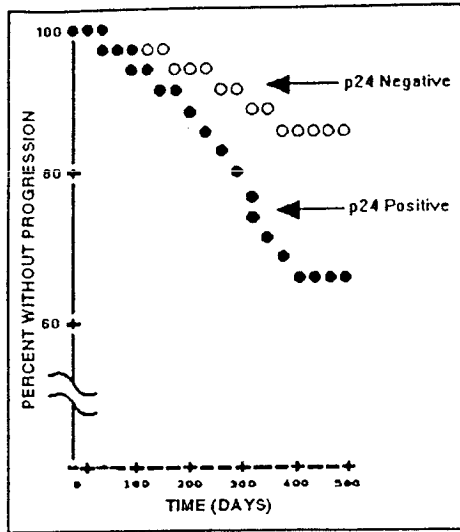
Figure 3 Panel B shows the Kaplan-Meier curves for asymptomatic subjects from the time of their initial test, irrespective of the time a subject first tested positive for HIV-1 p24 antigen. Although the rate of disease progression for the 38 subjects who tested positive tended to be greater than that of the 121 subjects who tested negative for HIV-1 p24 antigen, the difference was not significant (p values were 0.15 by Wilcoxon and 0.075 by log rank statistical tests). This analysis illustrates that the clinical value of the HIV-1 p24 antigen test for prognosis or monitoring of asymptomatic subjects is evident only after a positive HIV-1 p24 antigen test result is obtained, which may require repeated testing over time.

##### B. ARC subjects (CDC III)

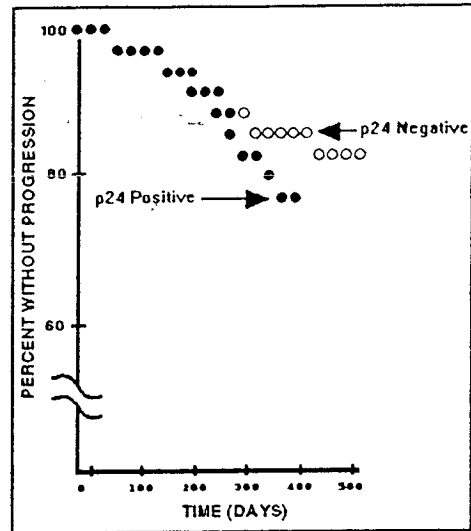
The rate of disease progression shown in Figure 4 for 29 ARC subjects who were HIV-1 p24 antigen positive, tended to be greater than that of the 112 ARC subjects who were negative for HIV-1 p24 antigen, but the difference was not statistically significant (p values were >0.15 by Wilcoxon, log rank and log-likelihood statistical tests). Therefore, based on the data obtained in this study, there was only a trend to suggest that the antigen test may be useful as a prognostic indicator for ARC subjects.

Figure 3. The Relationship Between HIV-1 p24 Antigen Test Results and Disease Progression for Asymptomatic Subjects

Panel A. Disease progression after a positive HIV-1 p24 antigen test result

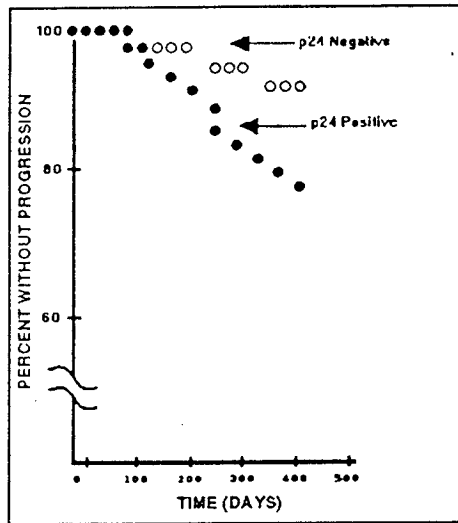


Panel B. Disease progression over the first 500 days



Progression is defined as reclassification to ARC (CDC III) or AIDS (CDC IV). (O) represents data for subjects who tested negative for HIV-1 p24 antigen throughout the study and (●) represents data for subjects who tested positive for HIV-1 p24 antigen at some time during the study. The survival curve in panel A is based on the data obtained subsequent to the first positive test result. The survival curve in Panel B is based on the data obtained over the first 500 days, irrespective of the time of the first positive test result.

Figure 4. The Relationship Between HIV-1 p24 Antigen Test Results and Disease Progression for ARC Subjects After a Positive HIV-1 p24 Antigen Test Result



Progression is defined as reclassification to AIDS (CDC IV). (●) represents data for subjects who tested negative for HIV-1 p24 antigen throughout the study and (O) represents data for subjects who tested positive for HIV-1 p24 antigen at some point during the study. The survival curve is based on data obtained for subjects after their first positive HIV-1 p24 antigen test.

**C. Relationship Between CD4+ Cell Counts and HIV-1 p24 Antigen**

Clinical studies show there is a close association between CD4 lymphocyte counts and disease severity. CDC's revised classification system for HIV infection and expanded AIDS surveillance case definition for adolescents and adults is based, in part, on CD4 lymphocyte counts. The relationship between CD4 lymphocyte counts and the presence of HIV-1 p24 antigen in blood specimens obtained from 569 patients was studied by independent investigators who collected specimens during routine medical evaluations of disease status. Specimens were analyzed for CD4 counts and tested for the presence of HIV-1 p24 antigen using the COULTER HIV-1 p24 Antigen Assay and Neutralization test. The proportion of patients with and without detectable HIV-1 p24 antigen are presented in Table 10 as a function of the patients' CD4 lymphocyte counts. The data shows that the proportion of patients who were positive for HIV-1 p24 antigen increased as the CD4 lymphocyte count decreased. These results are consistent with the observed prevalence of detectable HIV-1 p24 antigen in various HIV-1 clinical groups and the prognostic implications described in previous sections.

Table 10. Presence of HIV-1 p24 Antigen as a Function of CD4 Lymphocyte Counts in Blood of HIV-1-infected Patients

p24 Antigen Test Result	CD4 Lymphocyte Counts		
	≤ 200 Number of Patients (%)	201-500 Number of Patients (%)	> 500 Number of Patients (%)
p24 Antigen Positive	42 (63)	81 (56)	154 (43)
p24 Antigen Negative	25 (37)	63 (44)	204 (57)

V. PERFORMANCE CHARACTERISTICS OF THE NEUTRALIZATION TEST

A. Performance with repeatedly reactive specimens

Table 11 summarizes the performance of the COULTER HIV-1 p24 Antigen Neutralization test with specimens obtained during the clinical trials for subjects who were repeatedly reactive in the COULTER HIV-1 p24 Antigen Assay.

Table 11. Performance of the COULTER HIV-1 p24 Antigen Neutralization Kit with Repeatedly Reactive Specimens

Subjects	Number Screened	Number Repeatedly Reactive	Not Neutralized (Valid Test)	Indeterminate (Invalid Test)	(Neutralized (%))
Donors	301,699	33 <sup>a</sup>	31 <sup>b</sup>	0	2 (0.00066) <sup>c</sup>
Asymptomatic (CDCII) <sup>d</sup>	505	108	1 <sup>e</sup>	10 <sup>f</sup>	97 (19.2)
ARC (CDCIII) <sup>d</sup>	364	182	3 <sup>e</sup>	5 <sup>f</sup>	174 (47.8)
AIDS (CDCIV) <sup>d</sup>	485	276	0	12 <sup>f</sup>	264 (54.4)
Unknown Classification <sup>d</sup>	61	43	0	0	43 (70.5)
CD <sub>4</sub> > 500 <sup>g</sup>	358	154	0	0	154 (43.0)
CD <sub>4</sub> 201-500 <sup>g</sup>	144	81	0	0	81 (56.3)
CD <sub>4</sub> ≤ 200 <sup>g</sup>	67	42	0	0	42 (62.7)

a AAB8 Central laboratory repeatedly reactive specimens, as described in Table 3

b Specimens were negative for HIV-1 by Western blot and DNA-PCR analyses.

c Specimens were positive for HIV-1 antibody by Western blot analysis.

d Includes data from Table 5, Table 8 and Table 9.

e Neutralization tests met assay criteria, but specimens did not neutralize. After treatment with COULTER's investigational ICD reagent to dissociate antigen-antibody complexes, three specimens neutralized in the HIV-1 p24 Antigen Neutralization Test. One specimen was not treated with ICD.

f Specimens contained low levels of HIV-1 p24 antigen. Four of the 27 specimens (One CDC II, one CDC III and two CDC IV) were neutralized after being treated with COULTER's investigational ICD reagent to dissociate antigen-antibody complexes, and 23 specimens were not treated with ICD reagent.

g Includes data from Table 8 and Table 9.

h Includes data from Table 10.

Based on an assumed zero prevalence of HIV-1 infection for donor populations in the absence of additional evidence of infection, the neutralization test was specific for HIV-1 antigen in the repeatedly reactive specimens obtained from normal blood donors. The neutralization test also detected HIV-1 p24 antigen in two specimens from random blood donors who were also positive for HIV-1 antibodies by Western blot analysis.

Among the 1,984 specimens of antibody positive subjects summarized in Table 11, 886 (44.6%) were repeatedly reactive and 855 (96.5%) of these were neutralized. There were four repeatedly reactive specimens that had a valid neutralization test, but did not neutralize by more than 40%. These were tested over a 15 to 57 day period of time. Four specimens had low levels of HIV-1 p24 antigen. Three of these four specimens were neutralized after being treated with COULTER's investigational ICD reagent to dissociate antigen-antibody complexes, and one specimen (OD <0.120 in the screening assay) was not treated with ICD reagent. There were 27 specimens that did not have a valid neutralization test. All 27 specimens had low levels of HIV-1 p24 antigen in the screening assay (22% were tested > 6 days, 37% were tested > 7 days and 42% were tested at or ≥ 8 days). Four of the 27 specimens were neutralized after being treated with COULTER's investigational ICD reagent to dissociate antigen-antibody complexes, and 23 specimens were not treated with ICD reagent.

Based on an assumed 100% prevalence in antibody positive individuals, the sensitivity of the neutralization test was 99.5% (855/859) for repeatedly reactive specimens with a valid neutralization test and 100% (855/855) for specimens that were stored and tested according to recommendations.

B. Analytical Sensitivity of the Neutralization Test

The sensitivity of the Neutralization test was evaluated for clinical trial specimens from patients who were antibody positive and repeatedly reactive in the HIV-1 p24 Antigen Assay. Based on the concentration of HIV-1 p24 antigen determined from the quantitative HIV-1 p24 antigen assay, there were 67 specimens among the clinical specimens tested that had very low concentrations of HIV-1 p24 antigen, from 15 pg/mL (mean OD<sub>450/570 nm</sub> 0.165) down to 4.8 pg/mL (mean OD<sub>450/570 nm</sub> 0.100). Ten of these 67 specimens had concentrations below the lowest Calibration Standard, 7.8 pg/mL (mean OD<sub>450/570 nm</sub> 0.10). All 67 specimens were neutralized in the Neutralization assay to a similar extent as specimens containing high concentrations of HIV-1 p24 antigen. These results suggest that the Neutralization test is at least as sensitive as the HIV-1 p24 Antigen Assay for HIV-1 p24 antigen.

C. Reproducibility of the HIV-1 p24 Neutralization Test

The reproducibility of the COULTER HIV-1 p24 Neutralization test was determined with the coded panel of paired fresh and frozen specimens as part of the clinical study. Table 12 shows the test results based on 24 determinations for these panel members.

Table 12. Reproducibility of the Neutralization Test for HIV-1 p24 Antigen Using Fresh and Frozen Specimens

Panel Member	Condition	Mean Absorbance Specimen + NNC OD <sub>450/750 nm</sub>	Mean Absorbance Specimen + NR OD <sub>450/750 nm</sub>	% Signal Reduction <sup>a</sup>	SD	%CV
1	Fresh	0.693	0.056	94.4	0.01	1.94
	Frozen	0.705	0.059	93.6	0.02	2.06
2	Fresh	0.543	0.050	94.1	0.01	0.89
	Frozen	0.544	0.073	92.6	0.03	3.33
3	Fresh	0.765	0.070	93.6	0.01	0.93
	Frozen	0.770	0.070	92.6	0.02	2.49
4	Fresh	0.506	0.050	93.7	0.01	1.33
	Frozen	0.530	0.052	92.7	0.04	3.82
5	Fresh	0.572	0.054	93.6	0.02	1.69
	Frozen	0.534	0.053	92.7	0.03	3.45
6	Fresh	0.172	0.033	91.0	0.04	3.75
	Frozen	0.176	0.033	89.0	0.09	9.68

Abbreviations: OD, optical density; NNC, Negative Neutralizing Control; NR, Neutralizing Reagent; SD, standard deviation; %CV, percent coefficient of variation.

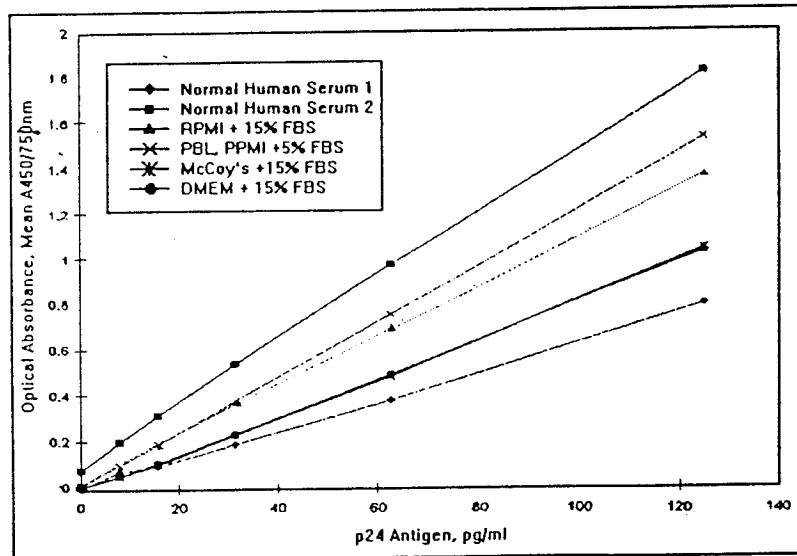
a % signal reduction is calculated using the formula described in the Results section of the COULTER HIV-1 p24 Antigen Neutralization Kit package insert.

#### VI. PERFORMANCE CHARACTERISTICS OF THE QUANTITATIVE ASSAY USING SERUM, PLASMA, OR TISSUE CULTURE SUPERNATANTS

##### A. Calibration Curves

Quantitation of HIV-1 p24 antigen using the COULTER HIV-1 p24 Antigen Assay was demonstrated for commonly used culture media and peripheral blood lymphocyte (PBL) cultures. Representative Calibration Curves are shown in Figure 5 for HIV-1 p24 Antigen Reagent diluted in RPMI, McCoy's and Dulbecco's MEM media containing 15% fetal bovine serum (FBS) and PBL culture supernatants containing 5% FBS. Similar Calibration Curves were obtained for media containing 5%, 10%, or 20% FBS (data not shown) and normal human serum. The lowest and the highest calibration curve obtained during the clinical trials for normal human serum are shown in Figure 5 to illustrate the widest range over which Calibration Curves for a specific media may vary.

Figure 5. COULTER HIV-1 p24 Antigen Assay Calibration Curves



The mean and range of absorbance values that can be expected for each recommended dilution of the COULTER HIV-1 p24 Antigen Reagent (Calibration Standard) are shown in Table 13. Table 13 includes the data for thirty Calibration Curves using Calibration Standards prepared in various tissue culture media containing 5-20% FBS, human serum and PBL culture supernatants and then tested in duplicate. The slopes for the Calibration Curves ranged from 0.006 to 0.016 (mean = 0.009). Regression analysis of individual Calibration Curves showed excellent correlation among the dilutions tested, such that correlation coefficients (r) for the thirty curves ranged between 0.995 and 1.00.

Table 13. Expected Range of Absorbance Values for HIV-1 P24 Antigen Assay Calibration Standards

P24 Antigen Calibration Standard pg/mL	Expected Absorbance Mean OD <sub>450nm</sub>	Expected Range of Absorbance Values	
		lower	upper
0.0	0.022	0.000	0.058
7.8	0.100	0.051	0.201
15.6	0.175	0.097	0.318
31.3	0.325	0.194	0.542
62.5	0.629	0.387	0.984
125.0	1.213	0.811	1.830

**B. Quantitation of p24 Antigen in Clinical Trial Specimens**

As part of the multicenter clinical trial, COULTER's quantitative assay for HIV-1 p24 antigen was evaluated for a single serum or plasma specimen collected from 590 subjects without regard for disease classification. Table 14 shows the mean concentration of HIV-1 p24 antigen determined for an initial and a repeat test of the clinical specimens and their frequency of distribution across the range of HIV-1 p24 antigen concentrations represented by the Calibration Standards.

Table 14. Quantitation of HIV-1 P24 Antigen in Clinical Specimens

HIV-1 p24 Antigen Concentration Range	Mean p24 Antigen Initial Test	Concentration (pg/ml) Repeat Test	Number of Subjects (%)
<7.8 pg/mL (below curve)	5.0	17.4	4 (0.7)
7.8-15.6 pg/mL	12.0	13.5	65 (11.0)
15.7-31.3 pg/mL	23.3	24.5	167 (28.3)
31.4-62.5 pg/mL	43.5	39.9	157 (26.6)
62.6-125 pg/mL	86.7	83.1	122 (20.7)
>125 pg/mL (above curve)	199.2	184.3	75 (12.7)

The concentration of HIV-1 p24 antigen in 86.4% (511/590) of the clinical specimens tested was within the range of the Calibration Curve on the initial and the repeat test. The correlation between the initial estimate for the concentration of HIV-1 p24 antigen and the estimate on the repeat test was  $r$  (correlation coefficient) = 0.91. All of the clinical specimens produced absorbance values greater than the cutoff value. All of the specimens were neutralized in the supplemental HIV-1 p24 Neutralization test. For specimens that produce absorbance values above the range of the Calibration Curve, additional information about the concentration of HIV-1 p24 antigen may be determined by testing diluted specimens. It should be noted that quantitation of some diluted specimens may not be possible due to complicated interactions between antibodies and antigens.

**C. PBL (Peripheral Blood Lymphocyte) Culture Supernatants**

The performance of the HIV-1 p24 antigen assay with supernatants from PBL cell cultures was studied. Supernatants were obtained from six PBL cultures that were co-cultured for seven days with PBLs from HIV-1-infected patients and tested in the HIV-1 p24 Antigen Assay and the HIV-1 p24 Antigen Neutralization test. HIV-1 culture supernatants often contain very high concentrations of p24 antigen, therefore, the culture supernatants were first diluted 1:200 in RPMI 1640 media containing 5% FBS, and then diluted in two-fold steps in the same media prior to testing for HIV-1 p24 antigen. Dilutions of PBL supernatants between 1:1600 to 1:25,600 brought test results for these specimens within the upper range of the Calibration Curve of the HIV-1 p24 Antigen Assay. Further dilutions of these specimens produced linear absorbance values from which the concentration of HIV-1 p24 antigen could be determined. Supernatants from all six PBL cultures were neutralized in the HIV-1 p24 Antigen Neutralization test.

**D. Specificity Using Cell Culture Supernatants**

The specificity of the quantitative assay for HIV-1 p24 antigen using tissue culture specimens was studied by testing supernatants of infected cells or cells co-cultured with various retroviruses and other infectious agents at a density of  $1 \times 10^6$  cells/mL. Quantitative tests were performed using supernatants from HUT 78 cells infected with SiVmac-251, H9 cells infected with HIV<sub>1-8</sub> or HIV-2 ROD and MoT cells infected with HTLV-II. Supernatants from similar cultures of HUT 102 (HTLV-I) cells, 8E5 cells and A3.01 cells were used as media for preparation of Calibration Standards and negative controls. Other virus/bacterial cultures studied included HTLV-I, HTLV-II, Cytomegalovirus, Herpes simplex type 1 (HSV-1), type 2 (HSV-2), Epstein-Barr virus and Chlamydia trachomatis (L2 serotype). High concentrations of P24 antigen were detected in tissue culture supernatants from HIV<sub>1-8</sub> and the 8E5 cells, and dilutions of supernatants produced linear assay results. HIV-2 and SiV culture supernatants were reactive in the assay, but the antigen levels were low and tests of diluted specimens were not linear, suggesting that antigens of these viruses cross-react to a limited extent in the HIV-1 p24 antigen assay. All other cell culture specimens tested in the assay were not reactive.

**E. Sensitivity and Specificity of the COULTER HIV-1 p24 Antigen Assay for HIV-1 Subtype O**

The ability of the COULTER HIV-1 p24 Antigen Assay to detect p24 antigens of HIV-1 Subtype O was investigated in an independent laboratory study. Lymphocytes from nine patients infected with HIV-1 Subtype O were co-cultured with PHA-stimulated PBLs and tested for p24 antigen daily. P24 antigen was detected in all nine of the tissue culture supernatants within seven days. The presence of p24 antigen was confirmed using the COULTER HIV-1 p24 Antigen Neutralization test.

**REFERENCES**

- Gottlieb MS, Schroff R, et al.: 1981. Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired immunodeficiency. N. Engl. J. Med: 305: 1425-1431.
- Harris C, Small CB, et al.: 1981. Immunodeficiency in female sexual partners of men with acquired immunodeficiency syndrome. N. Engl. J. Med: 308: 1181-1184.
- Centers for Disease Control. Pneumocystis carinii pneumonia among persons with hemophilia A. MMWR: 31: 365-367.
- Centers for Disease Control. Possible transfusion-associated acquired immune deficiency (AIDS)-California. MMWR: 31: 652-654.
- Centers for Disease Control. Unexplained immunodeficiency and opportunistic infection in infants-New York, New Jersey and California transfusion-associated acquired immune deficiency (AIDS)-California. MMWR: 31: 665-667.
- Salahuddin, SZ, Markham PD, Popovic M, Samgadhara MG, Orndoff S, Fladagar A, Patel S, Gold J and Gallo RC: 1985. Isolation of infectious human T-cell leukemia/lymphotropic virus type III (HTLV-III) from patients with acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC) and healthy carriers: a study of risk groups and tissue sources. Proc. Natl. Acad. Sci. USA: 82: 5530-5534.
- Lange JMA, Coutinho RA, Krone WJA, Verdonck LF, Danner SA, van der Noordaa J and Goudsmit J: 1986. Distinct IgG recognition patterns during progression of subclinical and clinical infection with lymphadenopathy associated virus/human T lymphotropic virus. Br Med J: Vol. 292: 228-230.
- Coutinho RA, Goudsmit J, Paul DA, de Wolf F, Lange JMA and van der Noordaa J: 1987. Dutch AIDS-study group, The natural history of HIV infection in homosexual men. Ann Inst Pasteur Virol: T38: 67-74.
- Ritter J, Escaich S, Trepo C and Sepetjan M. HIV antigen detection in antibody negative sera. Abstract 1627 International AIDS Congress.
- Casey JM, Kim Y, Andersen PR, Watson KF, Fox JL and Devare SG: 1985. Human T-cell lymphotropic virus type III: immunologic characterization and primary structure analysis of the major internal protein, p24. J Virology: 55: 417-423.
- Veronese FD, Samgadhara MG, Rahman R, Markham PD, Popovic M, Bodner AJ and Gallo RC: 1985. Monoclonal antibodies specific for p24, the major core protein of human T-cell leukemia virus type III. Natl Acad Sci USA: 82: 5199-5202.

12. Schupback J, Haller O, Vogt M, Ludyir R, Jolier H, Oritz G, Popovic M, Samigaouan H and Sano H: 1989. Anticorps to HIV pre-AIDS and in groups at risk for AIDS. N Engl J Med: 312: 265-270.
13. Pan L-Z, Cheng-Mayer C and Levy JA: 1987. Patterns of antibody response in individuals infected with the human immunodeficiency virus. J Infect Dis: 155: 626-632.
14. Enzensberger Doerr HW, Preiser W, Storkel F, Hach-Wunderle V and Scharrer I. Kinetics of HIV-antigen and antibodies in HIV infected haemophiliacs. Abstract 1623 International AIDS Congress.
15. Fenouillet E, Blanes N, Coutellier A and Gluckman JC. 1993. Relationship between anti-p24 antibody levels and p24 antigenemia in HIV -infected patients. AIDS Res. and Human Retroviruses: 9: 1251-1255.
16. Lange JMA, Paul DA, et al: 1987. Viral gene expression, antibody production and immune complex formation in human immunodeficiency virus infection. Long-term HIV-1 infection without immunologic progression. AIDS: 1; 15-20.
17. Fauci AS, Schnittman SM and Poli G: 1991. Immunopathogenic Mechanisms in Human Immunodeficiency Virus (HIV) Infection. Ann Int Med: 114; 678-693.
18. Lifson AR, Buchbinder SP, Sheppard HW, Mawle AC, Wiiber JC, Stanley M, Hart CE, Hessel NA and Holmberg SD: 1991. Long-term Human Immunodeficiency Virus Infection in Asymptomatic Homosexual and Bisexual Men with Normal CD4+ Lymphocyte Counts: Immunologic and Virologic Characteristics. J Infect Dis: 163; 959-965.
19. Buchbinder SP, Katz MH, Hessel NA, O'Malley PM, and Holmberg SD: 1994. AIDS: 8; 1123- 1128.
20. Fenouillet E, Blanes N, Coutellier A and Gluckman JC. 1993. Relationship between anti-p24 antibody levels and p24 antigenemia in HIV -infected patients. AIDS Res. and Human Retroviruses: 9; 1251-1255.
21. Burgard M, Mayaux MJ, Blanche S et al. 1992. The use of viral culture and p24 antigen screening to diagnose human immunodeficiency virus infection in neonates. N. Engl. J. Med: 327; 1192-1197.
22. Resnick L, Veren K, Salahuddin SZ et al. 1986. Stability and inactivation of HTLV-III/LAV under clinical and laboratory environments. JAMA: 255; 1887-1891.
23. Bond WW, Favero MS, Peterson NJ, and Ebert JW. 1983. Inactivation of hepatitis B virus by intermediate-to-high level disinfectant chemicals. J. Clin. Microbiol: 18; 535-538.
24. National Committee for Clinical Laboratory Standards. Approved guideline: Preparation and testing of reagent water in the clinical laboratory. 2nd e. Villanova, PA: National committee for Clinical Laboratory Standards, 1991;11(9). (NCCLS document I/LA 18-P).
25. Alter HJ, Epstein JS, Swenson SJ, Van Räden MJ, Ward JW, Kaslow RA, Menitove JE, Klein HG, Sandler SG, Sayers MH, Hewlett IK and Chernoff AI. 1990. Prevalence of human immunodeficiency virus type 1 p24 antigen in U. S. blood donors- An Assessment of the Efficacy of Testing In Donor Screening. N. Engl. J. Med: 323; 1312-1317.
26. Centers for Disease Control. 1986. Classification System for Human T-Lymphotropic Virus Type III/ Lymphadenopathy-Associated Infections. (MMWR): 35; 334-339
27. Centers for Disease Control. 1992. Revised Classification System for HIV Infection and Expanded AIDS Surveillance Case Definition for Adolescents and Adults, Draft published in 1992. MMWR; November 15, 1991); 41:1-17.
28. SAS Institute, Inc. (1988). SAS Technical Report: P-179, Additional SAS/STAT Procedures, Release 6.03.

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