





ANTIBODY TO HUMAN IMMUNODEFICIENCY VIRUS TYPE 1

HIVAG™-1 Monoclonal

NAME AND INTENDED USE

THE  HIVAG™-1 MONOCLONAL KIT IS A QUALITATIVE *IN VITRO* ENZYME IMMUNOASSAY FOR THE DETECTION OF UNCOMPLEXED HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1) p24 ANTIGEN IN HUMAN SERUM OR PLASMA. THE  HIVAG-1 MONOCLONAL IS INTENDED TO BE USED AS A SCREEN FOR DONATED BLOOD AND PLASMA AND AS AN AID IN THE DIAGNOSIS AND PROGNOSIS OF HIV-1 INFECTION.

THE  HIVAG™-1 MONOCLONAL BLOCKING ANTIBODY KIT (SOLD SEPARATELY) IS INTENDED TO BE USED WITH THE REAGENTS SUPPLIED WITH THE  HIVAG™-1 MONOCLONAL KIT AS A QUALITATIVE, ADDITIONAL, MORE SPECIFIC TEST FOR THE DETECTION OF HIV-1 p24 ANTIGEN IN PLASMA AND SERUM.

WARNING: NOT INTENDED FOR USE WITH CELL CULTURE SUPERNATANTS.

66-5980/R13

WARNING: A SOFTWARE UPGRADE AND/OR PROTOCOL EDITS WILL BE REQUIRED PRIOR TO IMPLEMENTING THIS ASSAY. CONTACT YOUR ABBOTT REPRESENTATIVE.

 ABBOTT LABORATORIES
Diagnostics Division

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NAME AND INTENDED USE

⊖ HIVAG™-1 Monoclonal Kit is a qualitative *In Vitro* enzyme immunoassay for the detection of uncomplexed Human Immunodeficiency Virus Type 1 (HIV-1) p24 antigen in human serum or plasma. The ⊖ HIVAG-1 Monoclonal is intended to be used as a screen for donated blood and plasma and as an aid in the diagnosis and prognosis of HIV-1 infection.

THE ⊖ HIVAG™-1 MONOCLONAL BLOCKING ANTIBODY KIT (SOLD SEPARATELY) IS INTENDED TO BE USED WITH THE REAGENTS SUPPLIED WITH THE ⊖ HIVAG™-1 MONOCLONAL KIT AS A QUALITATIVE, ADDITIONAL, MORE SPECIFIC TEST FOR THE DETECTION OF HIV-1 p24 ANTIGEN IN PLASMA AND SERUM.

WARNING: NOT INTENDED FOR USE WITH CELL CULTURE SUPERNATANTS.

SUMMARY AND EXPLANATION OF THE TEST

The etiologic agent of the Acquired Immune Deficiency Syndrome (AIDS) is a retrovirus called Human Immunodeficiency Virus (HIV).^{1,2} It was first isolated from AIDS patients,^{3,4} and subsequently from infected subjects at all stages of infection.⁵ HIV can be transmitted by exposure to blood (including sharing of contaminated needles and syringes) and certain blood products, sexual contact or from an infected mother to her child.^{6,7,9}

HIV infection is usually identified indirectly by the presence of specific antibody in serum or plasma.¹⁰ Direct evidence for HIV-1 infection can be obtained by culturing plasma or serum and detecting cellular (DNA) or genomic (RNA) nucleic acid sequences, or viral p24 antigen.¹¹ While cell culture can identify infectious virus in most people at all stages of infection,^{12,13} the rate of virus isolation by plasma culture varies according to clinical stage.^{14,15} Amplification of nucleic acids by polymerase chain reaction (PCR) allows detection of HIV-1 at all stages of infection in most, although not all, infected subjects.^{16,21} Detection of p24 antigen in serum or plasma varies with the clinical stage of infection.^{14,22,23}

During the primary stage of HIV-1 infection, p24 antigen may be present in individuals for a short time before antibody becomes detectable.^{24,28} Various reports have documented this finding in blood, plasma and tissue donors,^{29,34} seronegative emergency department patients,³⁵ and high risk individuals who seroconverted.^{36,39}

In seropositive subjects, the rate of p24 antigen detection is more frequent in subjects with AIDS and AIDS-related complex (ARC) than in asymptomatic individuals.^{15,22,23} In addition, subjects with persistent p24 antigenemia following seroconversion have a more rapid progression to AIDS than antigen negative subjects.⁴²⁻⁴⁵ Therefore, p24 antigen is a useful prognostic marker of clinical progression.^{46,47}

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

⊖ HIVAG-1 Monoclonal is a sandwich solid phase enzyme immunoassay used to detect HIV-1 p24 antigen. HIV-1 virions, when present in the test sample, are disrupted by the addition of Specimen Diluent containing Triton® X-100.

Beads coated with monoclonal antibody (Ab) to HIV-1 p24 are incubated with the samples (Controls and specimens). HIV-1 p24 antigen (Ag) present in the sample binds to the monoclonal antibody (Ab) on the bead. Unbound materials are removed by washing the beads.

Rabbit antibody (Ab) to HIV-1 is then incubated with the beads and, if Ag is present in the sample, the rabbit Ab binds to the Ag on the bead. Unbound materials are removed by washing the beads. Goat antibody to rabbit IgG conjugated with horseradish peroxidase (anti-rabbit IgG:HRPO) is then incubated with the beads, and binds to the rabbit Ab on the bead.

Unbound conjugate is removed by washing the beads. The beads are then incubated with o-Phenylenediamine (OPD) Substrate Solution containing hydrogen peroxide. The reaction of OPD Substrate Solution with HRPO yields a yellow-orange color. The intensity of the color formed is proportional to the amount of uncomplexed HIV-1 p24 Ag in the sample. The enzyme reaction is stopped by the addition of 1 N Sulfuric Acid and the intensity of color developed is read using a spectrophotometer set at 492 nm (A_{492}).

Specimens with absorbance values less than the Cutoff Value are negative by ⊖ HIVAG-1 Monoclonal. Specimens with absorbance values equal to or greater than the Cutoff Value are considered reactive, and must be retested in duplicate to determine whether the reactivity is a reproducible finding. Repeatedly reactive specimens should be tested using ⊖ HIVAG-1 Monoclonal BLOCKING ANTIBODY. This procedure uses a specific antibody neutralization step followed by testing with ⊖ HIVAG-1 Monoclonal to indicate the presence of uncomplexed HIV-1 p24 antigen. Specimens which are neutralized in this procedure are considered positive for HIV-1 p24 antigen. Specimens which are not neutralized in this procedure are considered indeterminate.

REAGENTS

No. 2A81, ⊖ HIVAG-1 Monoclonal Kit (100/1000 tests)

- ① 1 Vial (100)/2 Vials (500 each) Antibody to HIV-1 p24 (Monoclonal) Coated Beads.
- ② 1 Vial (27 mL)/2 Vials (110 mL each) Anti-Rabbit IgG Conjugate. Antibody to Rabbit IgG (Goat): Peroxidase (Horseradish). Minimum concentration: 0.01 µg/mL. Preservatives: 0.01% Gentamicin and 0.10% ProClin™ 300.
- ③ 1 Vial (6.5 mL)/2 Vials (6.5 mL each) Positive Control. HIV-1 Antigen(s) in TRIS Buffered Saline, Inactivated. Preservative: 0.1% Sodium Azide.
- ④ 1 Vial (9.5 mL)/2 Vials (9.5 mL each) Negative Control. Recalcified Human Plasma in TRIS Buffered Saline. Nonreactive for HIV-1 Antigen, HBsAg, anti-HIV-1/HIV-2 and anti-HCV. Preservative: 0.1% Sodium Azide.
- ⑤ 1 Vial (27 mL)/2 Vials (110 mL each) Antibody to HIV-1 (Rabbit). Minimum concentration: 0.1 µg/mL. Preservative: 0.1% Sodium Azide.
- ⑥ 1 Vial (100 mL)/2 Vials (100 mL each) Specimen Diluent. Preservative: 0.1% Sodium Azide.
- ⑦ 1 Bottle (10 Tablets)/2 Bottles (40 Tablets each) OPD (o-Phenylenediamine • 2HCl) Tablets. OPD/Tablet: 12.8 mg.
- ⑧ 1 Bottle (55 mL)/2 Bottles (220 mL each) Diluent for OPD (o-Phenylenediamine • 2 HCl). Citrate-Phosphate Buffer containing 0.02% Hydrogen Peroxide.

* There are no components 7 and 8.

The stopping reagent is provided as an accessory to the ⊖ HIVAG-1 Monoclonal Kit and consists of:

- ① 1 N Sulfuric Acid, No. 7212 (Most U.S. and International Locations).

Use of acid other than that supplied by ABBOTT may result in instability of the developed color. To be suitable as a stopping reagent, Sulfuric Acid must pass the following test each time it is prepared. The following test cannot be performed on the COMMANDER® system. Use a Quantum™ II, Quantumatic™, or suitable spectrophotometer to perform this test.

1. Pipette 300 µL of OPD Substrate Solution into 5 EIA assay tubes or acid washed/distilled or deionized water rinsed tubes.
2. Add 1 mL of the 1 N Sulfuric Acid under test to each of the five tubes.
3. Measure the A_{492} of the OPD/Acid Solution against distilled or deionized water at "0 TIME" and "120 MIN".

4. Calculate the Mean Absorbance at "0 TIME" and "120 MIN".

5. To be acceptable, acid must exhibit:

- a. an A_{492} of less than 0.040 at "0 TIME" and
- b. a difference of less than 0.030 units in the values obtained at "0 TIME" and "120 MIN".

⊖ HIVAG-1 Monoclonal BLOCKING ANTIBODY (No. 4A30) is available as an accessory to the ⊖ HIVAG-1 Monoclonal Kit:

No. 4A30, ⊖ HIVAG-1 Monoclonal BLOCKING ANTIBODY (25 tests)

- ① 1 Vial (3 mL) Blocking Antibody to HIV-1 (Human), Inactivated. (Solution A). Minimum liter: 1:10 Nonreactive for HBsAg and anti-HCV. Preservative: 0.1% Sodium Azide.
- ② 1 Vial (5 mL) Control Reagent. (Solution B). Recalcified Human Plasma. Nonreactive for HIV-1 Antigen, HBsAg, anti-HIV-1/HIV-2 and anti-HCV. Preservative: 0.1% Sodium Azide.

WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE

Safety Precautions

CAUTION: This kit contains human sourced components. No known test method can offer complete assurance that products derived from human sources will not transmit infection. Therefore, all human sourced material should be considered potentially infectious. It is recommended that all specimens and kit reagents be handled in accordance with Biosafety Level 2 practices as described in the CDC NIH publication, *Biosafety in Microbiological and Biomedical Laboratories*⁴⁸ or other equivalent guidelines.^{49,50} The Positive Control has been inactivated by sonication and detergent treatment.

WARNING: FDA has licensed this test kit for use with human serum or plasma 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.

1. All human specimens and kit reagents should be handled in accordance with the biosafety practices as specified in the OSHA Standard on biohazardous pathogens or other equivalent biosafety guidelines.^{48,49,51} These precautions include, but are not limited to the following:
 - Wear gloves when handling specimens or reagents.
 - Do not pipette by mouth.
 - Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where these materials are handled.
 - Clean and disinfect all spills of specimens and reagents using a suitable disinfectant⁵² such as 1% Sodium Hypochlorite.⁵³
2. Clean up spills of potentially infectious materials in accordance with established biosafety practices. A generally accepted procedure for cleaning such spills is to absorb the spill with toweling or other absorbent material, wipe the area with a detergent solution, and then wipe the area with an appropriate tuberculocidal disinfectant such as 0.5% Sodium Hypochlorite.⁵⁴
3. Dispose of all materials that have come into contact with specimens and reagents in accordance with local, state and federal regulations.^{55,56} Generally accepted procedures for the treatment of solid, potentially infectious wastes include incineration or autoclaving. Sharps, such as contaminated probes, should be placed in a puncture-resistant container prior to treatment and/or disposal. Liquid wastes containing acid should be neutralized prior to the addition of a disinfectant or disposal. The addition of a disinfectant to the waste container helps to inactivate the infectious organisms that may be collected in the waste and thus reduce the risk to personnel who have to handle this material. Sodium Hypochlorite and glutaraldehyde solutions have been shown to be effective in inactivating organisms such as HBV, HCV and HIV, and can be used for this purpose. Appropriate personal protective equipment should be worn when these materials are handled.
4. Avoid contact of OPD and Sulfuric Acid with skin and mucous membranes. If these reagents come into contact with skin, wash thoroughly with cool water.
5. This product contains Sodium Azide as a preservative. Sodium Azide has been reported to form lead or copper azide in laboratory plumbing. These azides may explode on percussion, such as hammering. To prevent formation of lead or copper azide, thoroughly flush drains with water after disposing of solutions containing Sodium Azide. To remove contamination from old drains suspected of azide accumulation, the National Institute for Occupational Safety and Health (USA) recommends the following: (1) siphon liquid from drain trap using a rubber or plastic hose, (2) fill with 10% Sodium Hydroxide solution, (3) allow to stand for 16 hours, and (4) flush well with water.

Handling Precautions

1. Do not use kits beyond their expiration dates.
2. Do not mix reagents from different lots. Any OPD Tablet, Diluent for OPD, or Sulfuric Acid lot, may be used with any ⊖ HIVAG-1 Monoclonal kit lot.
3. Do not expose OPD reagents to strong light during incubation or storage.
4. Avoid contact of the OPD Substrate Solution and 1 N Sulfuric Acid with any oxidizing agent. Do not allow Substrate Solution or 1 N Sulfuric Acid to come in contact with any metal parts. Prior to use, thoroughly rinse glassware used for OPD Solution with 1 N Sulfuric Acid using approximately 10% of the container volume. Follow with three washes of distilled water at the same volume.
5. Avoid microbial contamination of reagents and water used for washing. Use of disposable pipette tips is recommended.
6. If the desiccant obstructs the flow of beads, remove from bead bottle prior to dispensing beads. Replace desiccant in bottle and tightly cap bottle for storage. Do not store beads with dispenser attached to bottle.
7. USE SEPARATE CLEAN DEDICATED DISPENSERS FOR THE ANTIBODY AND CONJUGATE SOLUTIONS AND FOR THE BLOCKING ANTIBODY TO AVOID CONTAMINATION.

INSTRUCTIONS FOR PREPARATION OF OPD SUBSTRATE SOLUTION

Bring OPD Reagents to room temperature (15 to 30°C).

CAUTION: Do not open OPD Tablet bottle until it is at room temperature.

At least 5 minutes, but not more than 60 minutes, prior to dispensing for Color Development, prepare the OPD Substrate Solution by dissolving the OPD (o-Phenylenediamine • 2HCl) Tablet(s) in Diluent for OPD. DO NOT USE A TABLET THAT IS NOT INTACT.

Using clean pipettes and metal-free containers (such as plastic ware or acid-washed and distilled/deionized water-rinsed glassware) follow the procedure below:

1. Transfer into a suitable container 5 mL of Diluent for OPD for each tablet to be dissolved.
2. Transfer appropriate number of OPD Tablets (see OPD Preparation Chart) into measured amount of Diluent for OPD using a nonmetallic forceps or equivalent. Return desiccant to bottle immediately, if removed to obtain a tablet, and close bottle tightly. Allow tablet(s) to dissolve. Do not cap or stopper the Substrate Solution bottle while the tablets are dissolving.

NOTE: The OPD Substrate Solution MUST be used within 60 minutes of preparation and MUST NOT be exposed to strong light. (Record the preparation time and expiration time of the OPD Substrate Solution.)

3. Just prior to dispensing for Color Development, swirl container gently to obtain a homogeneous solution. Remove air bubbles from dispenser tubing, and prime dispenser prior to use.

OPD PREPARATION CHART

No. Tests	Tablets	Diluent
13	1	5 mL
28	2	10 mL
43	3	15 mL
58	4	20 mL
73	5	25 mL
88	6	30 mL
103	7	35 mL
118	8	40 mL
133	9	45 mL
148	10	50 mL

NOTE: 300 μ L of OPD Substrate Solution is required for each specimen or Control as well as for each substrate blank. Laboratories using the COMMANDER[®] Parallel Processing Center (PPC) will require approximately an additional 3 mL of OPD Substrate Solution for instrument priming.

STORAGE INSTRUCTIONS

- Store kit reagents at 2 to 8°C. OPD Tablets and 1 N Sulfuric Acid may be stored at 2 to 30°C.
 - Kit reagents may be dispensed at room temperature or while cold. Return to 2 to 8°C storage immediately after use.
- CAUTION:** Do not open the OPD Tablet bottle until it is at room temperature (15 to 30°C).
- Retain desiccant in Bead bottle and in OPD Tablet bottle at all times during storage.
 - Reconstituted OPD Substrate Solution MUST be stored at room temperature and MUST be used within 60 minutes. Do not expose to strong light.
 - Replace desiccant in bead bottle, and tightly cap bottle for storage.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

The OPD Substrate Solution (OPD plus Diluent for OPD) should be colorless to pale yellow. A yellow-orange color indicates that the reagent has been contaminated and must be discarded. A value of less than 0.650 absorbance units for the difference between the Positive and Negative Control Means (P-N) may indicate technique errors or deterioration of the kit or OPD reagents. Such runs must be repeated.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

⊖ HIVAG[™]-1 Monoclonal and ⊖ HIVAG-1 Monoclonal BLOCKING ANTIBODY may be performed on human serum or plasma samples.

- Either serum (including serum collected in serum separator tubes) or plasma collected in EDTA, potassium oxalate, sodium heparin, sodium citrate, ACD, CPDA-1, CPD and CPD may be used in the test. The correct ratio of anticoagulant quantity to specimen volume as recommended by the manufacturer of anticoagulant is required. Remove the serum or plasma from the clot or red cells as soon as possible to avoid hemolysis. When possible, clear, nonhemolyzed specimens should be used.
- Specimens containing precipitate may give inconsistent test results. Such specimens should be clarified prior to assaying.
- Do not use heat-inactivated specimens.
- Performance has not been established using cell culture supernatants, cadaver specimens or body fluids other than serum or plasma, such as urine, saliva or pleural fluid.
- Assay performance was evaluated with negative specimens and samples made reactive by spiking with purified viral p24 and testing these samples after adding exogenous levels of bilirubin (up to 20 mg/dL), hemoglobin (up to 0.833 g/dL) or triglycerides (up to 1000 mg/dL). No qualitative performance differences were observed when testing these negative specimens and reactive samples.
- Due to the instability of the analyte, serum or plasma samples should be stored for no longer than seven days at a combination of room temperature (15 to 26°C) and nominal 4°C (2 to 8°C) following sample collection. This seven day period of storage should include no more than three days at room temperature. However, if storage periods greater than seven days are anticipated, the specimens should be removed from the clot or red blood cells, and the serum or plasma stored frozen at -20°C or colder. No more than five freeze-thaw cycles should be performed on any sample prior to testing for HIV-1 antigen.
- If specimens are to be shipped, they should be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and etiologic agents (42 CFR 72). Specimens may be shipped either refrigerated (2 to 8°C), on wet ice, frozen (-20°C or colder) on dry ice or at ambient temperatures that do not exceed 26°C.
- Specimens with obvious microbial contamination should not be used.
- All glassware or plastic materials coming into contact with the specimen should be free of any residue from previous specimens, reagents or cleaning compounds.

PROCEDURE

Materials Provided

No. 2A81-24 / 2A81-31, ⊖ HIVAG-1 Monoclonal Kit, 100/1000 Tests
(See REAGENTS for a complete listing)

The list of accessories required for the COMMANDER Flexible Pipetting Center (FPC) and Parallel Processing Center (PPC) are found in the appropriate COMMANDER Operations Manual(s). A combination of accessories is included with the COMMANDER FPC and PPC. ⊖ HIVAG-1 Monoclonal is designed to be compatible with the COMMANDER FPC and PPC. This product may be used with a Quantum[™] II or Quantumatic[™].

An optimum combination of the following accessories is provided for performance of the tests ordered:

- Reaction Trays
- Cover Seals
- Assay Tubes with Identifying Cartons
- 1 N Sulfuric Acid, No. 7212 (Most U.S. and International Locations)

Materials Required but not Provided

- Precision pipettes with disposable tips, EIA Pipetting Package, or similar equipment to deliver 20 μ L, 50 μ L, 200 μ L, 300 μ L (tolerance is \pm 5%) and 1 mL (tolerance is \pm 10%).
- Device for delivery of rinse solution (distilled or deionized water) such as Gorman Rupp[™] Dispensing Pump or equivalent.
- Qwikwash[®] or device for washing beads such as a Pentawash[®] or equivalent to deliver 4 to 6 mL per well, with a vacuum source such as a Gast[®] Vacuum Pump and a double trap for retaining the aspirate and maintaining minimum vacuum of 21 inches of mercury.
- The ProQuantum[™] Bead Washer and Reagent Dispenser may also be used in the performance of this assay.
- COMMANDER Dynamic Incubator (DI).
- Disposable, graduated pipettes or dispenser for measuring Diluent for OPD.
- Metal-free containers for the OPD Substrate Solution, can be plastic ware or acid-washed, distilled/deionized water-rinsed glassware.
- Protective gloves.
- Disinfectant as described in Safety Precautions.
- Nonmetallic forceps.
- COMMANDER PPC, Quantum II, Quantumatic or spectrophotometer capable of reading absorbances at 492 nm.
- Bead Dispenser.
- Test tubes and rack for dilution of specimens.
- Membrane Seal Puncture Tool for acid bottles.
- OPD Tray Covers (for COMMANDER testing).
- Blanking Beads (for COMMANDER testing).
- Distilled or deionized water.

* Included in EIA Pipetting Package

Additional Reagents Available

- Abbott OPD (o-Phenylenediamine • 2 HCl) Reagent, No. 6172.
- 1 N Sulfuric Acid, No. 7212. (Most U.S. and International Locations).
- ⊖ HIVAG-1 Monoclonal BLOCKING ANTIBODY, No. 4A30.

⊖ HIVAG-1 Monoclonal TEST PROCEDURE

Preliminary Comments

Laboratories using the COMMANDER Dynamic Incubator (DI), Flexible Pipetting Center (FPC) or Parallel Processing Center (PPC) refer to the appropriate COMMANDER Operations Manual(s) and note special COMMANDER instructions below. Precise timing of enzyme immunoassays is critical.

Follow the exact order of specimen and reagent addition as instructed in the steps of the ASSAY PROCEDURE.

- Assay three Negative Controls and two Positive Controls with each run of specimens. A substrate blank should be included each time the assay is run. Ensure that all reaction trays containing Controls and/or specimens are subjected to the same processing and incubation times. This may require maintenance of specific time intervals between processing trays. Once the assay has been started, complete all subsequent steps without interruption.

CAUTION: Use a separate disposable pipette tip for each specimen and Control in order to avoid cross-contamination.

- Identify the reaction tray wells for each Control and specimen.
- Dispense kit reagents at room temperature or while cold. Mix gently before using. Return reagents to 2 to 8°C storage immediately after use.
- Set the Dynamic Incubator to 40°C.
- After each step, visually verify the presence of solution and bead in each well.
- The exact order of specimen and reagent additions as described in this test procedure must be followed.

Procedural Notes

Sample Pipetting And Dilution

Assays performed on COMMANDER Parallel Processing Center (PPC), Quantum II and the Quantumatic may have the Specimen Diluent, Control and specimen pipetted using the Flexible Pipetting Center (FPC) with Assay Update Diskette Version 2.02 or higher.

A) When using the Flexible Pipetting Center Assay Update Diskette Version 2.02 use the appropriate assay protocol, HIVAG 1 PPC D0, HIVAG 1 QT D0, or HIVAG 1 ONB D0 for control or specimen dilution for the PPC or non-PPC processing (Quantum II or Quantumatic).

B) When using the Flexible Pipetting Center Assay Update Diskette Version 2.5 use the appropriate assay protocol, HIVAG 1 MC PPC D0 or HIVAG 1 MC QT D0 for control or specimen dilution for PPC or non-PPC processing (Quantum II or Quantumatic).

NOTE: When using FPC Assay Update Diskette Version 2.5 or higher, the FPC Assay Protocol for the QNB assay is not applicable.

When using FPC Assay Update Diskette Version 2.02 (LN6A97-21) or Version 2.5 (LN6A97-16) Rest of World Assay Update Diskettes, this assay's parameters for Assay List Number and Dispense Option must be changed. This assay must be pipetted using the Dilutor, position D0, with the Dil. Dispense option set to "POST" for each pipetting section. The list number must be 2A81.

Assay Selection On The PPC

- Insert tray and select the appropriate assay number for ⊖ HIVAG-1 MC. An operator-edited version may be used if the edited lines are consistent with the assay package insert specifications and are supported by documentation at the time of edit. Follow the instructions on the instrument display board.
When using an automated pipetting device, such as a COMMANDER Flexible Pipetting Center, verify that the correct PPC Assay Protocol has been selected for processing.

- Verify reagent dispenser assignment:

Station	Reagent	Dispenser Volume
2	Antibody	200 μ L
3	Conjugate	200 μ L
4	OPD Solution	300 μ L
5	Acid	300 μ L

- BLANKING (COMMANDER only)

NOTE: Use ABBOTT COMMANDER Reagent Blanking Beads only.

- During the conjugate incubation step, prepare a "blanks" tray using a separate tray. Place one blanking bead into each of the five wells, A1 through A5.

- At the conclusion of the conjugate incubation step, press the Blank key and insert the "blanks" tray, followed immediately by the first assay tray.
- At the conclusion of the OPD incubation step, insert the "blanks" tray prior to first assay tray of the batch.

General Notes

- Do not splash specimen, Antibody, or Conjugate outside of well or high up on well rim as it may not be removed in subsequent washings and may cause test interference.
- Verify that dispensing equipment delivers specified volume and appropriate dilutions for each procedure.
- When using a bead dispenser, remove cap from bead bottle, attach bead Dispenser and dispense beads into wells.
- Do not splash liquid while tapping trays.
- When washing beads, follow the directions provided with your washing apparatus to provide a total wash volume of 11 to 18 mL for each bead. Use distilled or deionized water.

COMMANDER® Dynamic Incubator

When using the COMMANDER Dynamic Incubator, select the ROTATION incubation method and the incubation temperature and time designated in the ASSAY PROCEDURE which follows. Use the ROTATION incubation method throughout the assay. Failure to use the Dynamic Incubator in the manner described in the Dynamic Incubator Operations Manual may result in incorrect assay results.

Color Development

- When transferring beads from wells to assay tubes, align inverted carton of tubes over their respective wells in the reaction tray. Press the tubes tightly over the wells and invert tray and tubes together so that beads fall into corresponding tubes. Blot excess water from top of tube carton.
 - Avoid strong light during Color Development.
 - Dispense acid in same sequence as OPD Substrate Solution.
 - Do not allow acid or OPD Substrate Solution to contact metal.
- NOTE:** Conjugate, Antibody and OPD Substrate dispenser must be rinsed with distilled or deionized water after each use. Refer to all dispenser inserts for cleaning procedure.

Reading (Quantum™ II or Quantumatic™)

- Remove air bubbles prior to reading absorbance.
- Visually inspect substrate blank tubes and discard those that are contaminated (indicated by a yellow-orange color). If both blanks are contaminated, the entire run must be repeated.
- Determine the absorbance of the substrate blank. In Mode 0, blank the instrument with the water tube using approximately 2 mL of distilled or deionized water and read the substrate blank as a sample. (Mode 0 refers to Mode 0 on Quantum II and Assay 0 on Quantumatic). Stop the Mode 0 assay. The absorbance value of the substrate blank relative to that of the water tube must be greater than or equal to -0.020 and less than or equal to 0.040 in order for the assay to be valid.
- If the substrate blank is valid, use it to blank the instrument. Read Negative and Positive Controls, then specimens.
- If the substrate blank is not valid, repeat Steps 3 and 4 using the alternate substrate blank. If the alternate substrate blank is invalid, the entire run must be repeated.
- If there is an interruption during the reading of samples, reblank the instrument with the substrate blank using the second substrate blank tube if necessary. Continue reading specimens.

ASSAY PROCEDURE FOR SERUM OR PLASMA

(See Preliminary Comments and Procedural Notes)

Laboratories using the COMMANDER Flexible Pipetting Center or Parallel Processing Center (PPC) should follow procedures in the appropriate COMMANDER Operations Manual(s). When using other automated instrumentation to deliver Controls and specimens ensure the instrumentation is compatible with this assay. Follow the manufacturer's directions to achieve the appropriate volumes and dilutions required. The following assay procedure should be used with the Quantum II, Quantumatic and PPC when pipetting manually. For color development on the PPC, refer to the PPC Operations Manual.

CAUTION: Verify that dispensing equipment delivers specified sample and/or reagent volumes and does not introduce cross-contamination.

FIRST INCUBATION

- Pipette 50 µL of Specimen Diluent into each reaction tray well to contain Controls or specimens.
- Pipette 200 µL of each Control into the appropriate reaction tray wells. (3 Negative Controls followed by 2 Positive Controls).
- Pipette 200 µL of specimen into the appropriate well.
- Gently tap trays.
- Add one Bead to each well containing a Control or specimen.
- Apply cover seal; tap tray gently to cover beads and remove any trapped air bubbles.
- Incubate at 38 to 42°C with ROTATION for 1 hour ± 5 minutes.
- Remove and discard cover seal. Wash each bead.

SECOND INCUBATION

- Add 200 µL of Antibody Solution to each reaction well.
- Apply new cover seal; tap tray gently.
- Incubate at 38 to 42°C with ROTATION for 1 hour ± 5 minutes.
- Remove and discard cover seal. Wash each bead.

THIRD INCUBATION

- Add 200 µL of Conjugate to each reaction well.
- Apply new cover seal; tap tray gently.
- Incubate at 38 to 42°C with ROTATION for 1 hour ± 5 minutes.
- Remove and discard cover seal. Wash each bead.

COLOR DEVELOPMENT

- Immediately transfer Beads to assay tubes.
- Prime OPD Dispenser immediately prior to dispensing OPD Substrate Solution.
- Pipette 300 µL of freshly prepared OPD Substrate Solution into two empty tubes (substrate blanks) and then into each tube containing a Bead.
- Cover and incubate at room temperature (15 to 30°C) for 28 to 32 minutes.
- Add 1 mL of 1 N Sulfuric Acid to each tube. (If necessary, agitate to mix.)
- Blank spectrophotometer with substrate blank at 492 nm.

- Determine absorbance of Controls and specimens (within 2 hours of addition of acid).

READING RESULTS FOR SERUM OR PLASMA

Performance of the \square HIVAG™-1 Monoclonal requires the use of a precision spectrophotometer (i.e., COMMANDER PPC, Quantum II or Quantumatic). REFER TO THE APPROPRIATE INSTRUMENT MANUAL FOR PROPER OPERATION AND CALIBRATION. SHOULD SOFTWARE NEED TO BE INSTALLED OR RELOADED, ANY EDITED ASSAY PROTOCOLS MUST BE RECREATED.

- Laboratories using the COMMANDER Parallel Processing Center (PPC) must use software version 6.00 or above. Laboratories using software versions 6.00, 6.01, 6.10, 6.11, 8.00, or 8.10 must create an edited Assay Protocol. An Abbott representative must initially make the assay protocol available on the software.

PPC version 6.00, 6.01, 6.10 & 6.11 users: Proceed to edit PPC Assay Protocol #15 to change the following parameters:

Line 02	Assay Name	"-A-HIVAG-1 MC 2A81"
Line 43	Neg. Min. Absorbance	"0.000"
Line 44	Neg. Max. Absorbance	"0.100"
Line 45	Negative Aberrant (%)	"35.0"
Line 49	Pos. Min. Absorbance	"0.800"
Line 51	Positive Aberrant (%)	"25.0"
Line 58	Min. Cntl. Diff. (P-N)	"0.650"
Line 66	Cutoff Offset	"0.040"

No other assay protocol parameters require edits. Verify that all other assay protocol parameters of the edited protocol match the assay protocol #15.

When using an automated pipetting device such as a COMMANDER Flexible Pipetting Center, ensure the edited PPC Assay Protocol number is assigned to the correct pipettor Test Number.

PPC version 8.00 & 8.10 users: Proceed to edit PPC Assay Protocol #30 to change the following parameters:

Line 02	Assay Name	"-A-HIVAG-1 MC 2A81"
Line 06	Assay Procedure	"LC"
Line 50	Neg. Min. Absorbance	"0.000"
Line 51	Neg. Max. Absorbance	"0.100"
Line 52	Negative Aberrant (%)	"35.0"
Line 57	Pos. Min. Absorbance	"0.800"
Line 59	Positive Aberrant (%)	"25.0"
Line 67	Min. Cntl. Diff. (P-N)	"0.650"
Line 73	Cutoff Offset	"0.040"

When pipetting with FPC Version 2.5 or higher, the Assay List Number and Assay Procedure Code must match that contained in the appropriate edited PPC Assay Protocol for \square HIVAG-1 Monoclonal. When configuring Assay Protocols in the FPC, ensure the assay procedure code is specified as "LC".

- Laboratories using the Quantum II should read this assay as follows: Laboratories using a Quantum II, Module A, List Number greater than 4045-97 should process \square HIVAG-1 Monoclonal using the Assay Protocol as provided in the software without editing.

Laboratories using the Quantum II, Module A, List Number 4045-96 or 97 must create an edited assay protocol. Edit Mode 1.21 as follows:

Edit Line 4: Negative Controls Minimum A to "0.000"
 Edit Line 5: Negative Controls Maximum A to "0.100"
 Edit Line 7: Negative Controls Aberrant Cutoff to "35.00"
 Edit Line 9: Positive Controls Minimum A to "0.800"
 Edit Line 11: Positive Controls Aberrant Value Option to "1"
 Edit Line 12: Positive Controls Aberrant Cutoff to "25.00"
 Edit Line 13: PC-NC Minimum Value to "0.650"
 Line 14 must be deleted (Delete by pressing 14 DASH CLEAR ENTER)
 Edit Line 21: Cutoff C to "0.040"

Save to new Mode

Verify that the edited protocol values and assay name match the protocol values and assay name below.

Name: -A-HIVAG-1 MC 2A81
 FILTERS = 492.600
 PATH LENGTH = 1.11
 NEGATIVE CONTROLS
 REPLICATION = 3
 MINIMUM ΔA = 0.000
 MAXIMUM ΔA = 0.100
 ABERRANT VALUE OPTION = 1
 ABERRANT CUTOFF = 35.00
 POSITIVE CONTROLS
 REPLICATION = 2
 MINIMUM ΔA = 0.800
 MAXIMUM ΔA = 1.999
 ABERRANT VALUE OPTION = 1
 ABERRANT CUTOFF = 25.00
 PC-NC
 MINIMUM VALUE = 0.650
 UNKNOWNNS
 REPLICATION = 1
 CUTOFF = A*NC+B*PC+C
 A = 1.000
 B = 0.000
 C = 0.040
 REACTIVE GRAY ZONE = 0.00%
 NEGATIVE GRAY ZONE = 0.00%
 DISTINCTION
 REACTIVE > = CUTOFF (0)
 REACTIVE < = CUTOFF (1)
 DISTINCTION = 0
 FLAGGING
 REACTIVE UNKNOWNNS (0)
 NEGATIVE UNKNOWNNS (1)
 FLAG = 0

3. Laboratories using the Quantumatic should read this assay as follows:
Laboratories using a Quantumatic, List Number greater than 7523-42, should process \square HIVAG-1 Monoclonal using the Assay Protocol as provided in the software without editing.

Laboratories using the Quantumatic, List Number 7523-42, must create an edited assay protocol. Edit an existing Cutoff Assay Protocol (e.g. Auszyme C) to include the following assay parameters. Verify that the edited protocol values and assay name match the protocol values and assay name below.

NAME: -A-HIVAG-1 MC 2A81
CUTOFF VALUE = (1.000)NC \bar{x} + (0.000)PC \bar{x} + 0.040
P>N
NO OF NEG CONTROLS = 3
MIN NEG CONTROL = 0.000
MAX NEG CONTROL = 0.100
NEG ABERRANT CUTOFF % = 35.0%
NO OF POS CONTROLS = 2
MIN POS CONTROL = 0.800
MAX POS CONTROL = 1.999
POS ABERRANT CUTOFF % = 25.0%
MIN CONTROL DIFF = 0.650
NO OF PATIENT REPL = 1
REACTIVE GRAY ZONE % = 0.0%
NEGATIVE GRAY ZONE % = 0.0%
NO OF POS-2 CONTROLS = 0

QUALITY CONTROL PROCEDURES FOR SERUM OR PLASMA

1. Substrate Blank Acceptance Criteria

- a. Quantum™ II/Quantumatic™ users: An assay run is considered valid with respect to the Substrate Blank if the blank has an absorbance value that is greater than or equal to -0.020 and less than or equal to 0.040. The determination of assay validity due to Substrate Blank must be done by the user. The Substrate Blank value is an indication of the integrity of the OPD Substrate Solution. If the Substrate Blank absorbance falls outside the acceptable range, the preparation of the substrate is in question and the alternate Substrate Blank may be used. If the alternate Substrate Blank falls outside the acceptable range, the assay is invalid, and the run must be repeated.
- b. COMMANDER® PPC users: Quality control with respect to the Substrate Blank is determined automatically by the COMMANDER Parallel Processing Center (PPC) according to the procedure described in the PPC Operations Manual. If the run is invalid, the preparation of the OPD Substrate Solution is in question and the run must be repeated.

NOTE: When a Quantum II, Quantumatic or Parallel Processing Center is used, refer to the appropriate Operations Manual to determine which calculations are performed automatically. If one of these instruments is not used, perform the following calculations on the assay data.

2. Negative Control Calculations and Acceptance Criteria

a. Calculation of Results

- 1) Calculation of Negative Control Mean Absorbance (NC \bar{x}).

Example:

Negative Control Sample No.	Absorbance
1	0.048
2	0.054
3	0.051
TOTAL	0.153

$$(NC\bar{x}) = \frac{\text{Total Absorbance}}{3} = \frac{0.153}{3} = 0.051$$

b. Negative Control Acceptance Criteria

Individual Negative Control Values must meet the following criteria:

- Individual Negative Control Values must be less than or equal to 0.100 and greater than or equal to 0.000.
- Individual Negative Control Values must be within the range 0.65 to 1.35 times the Negative Control Mean.
 - If one Negative Control Value does not meet either of the above criteria, it must be excluded as aberrant. The Negative Control mean must then be recalculated. All remaining individual Negative Control Values must meet the above criteria or the run is invalid and must be repeated.
 - If two values initially do not meet either of the above criteria, the test must be repeated.

3. Positive Control Calculations and Acceptance Criteria

a. Calculation of Results

- 1) Calculation of Positive Control Mean Absorbance (PC \bar{x}).

Example:

Positive Control Sample No.	Absorbance
1	1.445
2	1.481
TOTAL	2.926

$$(PC\bar{x}) = \frac{\text{Total Absorbance}}{2} = \frac{2.926}{2} = 1.463$$

b. Positive Control Acceptance Criteria

Individual Positive Control Values must meet the following criteria:

- Individual Positive Control Values must be less than or equal to 1.999 and greater than or equal to 0.800.
- Individual Positive Control Values must be within the range of 0.75 to 1.25 times the Positive Control mean.

4. Assay Run Validity Criteria

For the run to be valid, the difference between the mean absorbance of the Positive and Negative Controls (P-N) must be 0.650 or greater. If not, technique or deterioration of reagents may be suspect and the run must be repeated.

Calculations for Determining P-N

Example:

NC \bar{x} = 0.051

PC \bar{x} = 1.463

P-N = (1.463 - 0.051) = 1.412

RESULT CALCULATIONS FOR SERUM OR PLASMA

Calculation of the Cutoff Value

The Cutoff Value is the mean absorbance of the Negative Control plus 0.040.

Cutoff Value = NC \bar{x} + 0.040

Example:

NC \bar{x} = 0.051

Cutoff Value = 0.051 + 0.040 = 0.091

INTERPRETATION OF RESULTS FOR SERUM OR PLASMA

- Specimens with absorbance values less than the Cutoff Value are considered negative by the criteria of \square HIVAG™-1 Monoclonal.
- Specimens with absorbance values greater than or equal to the Cutoff Value are considered initially reactive by the criteria of \square HIVAG-1 Monoclonal but before interpretation, the original sample must be retested in duplicate with \square HIVAG-1 Monoclonal. If either duplicate retest is reactive, the specimen is considered to be repeatedly reactive for HIV-1 p24 antigen by the criteria of \square HIVAG-1 Monoclonal.
- Repeatedly reactive specimens must be further tested with \square HIVAG-1 Monoclonal BLOCKING ANTIBODY. Repeatedly reactive specimens that are neutralized in this procedure are considered positive for HIV-1 p24 antigen. Repeatedly reactive specimens that do not have a valid neutralization test, i.e. the mean S/CO of the specimen in the presence of \square HIVAG-1 Monoclonal BLOCKING ANTIBODY Control Reagent (solution B) is less than 1.0, are considered indeterminate. Indeterminate results should not be interpreted as positive or negative and should be followed-up by repeat testing the original specimen and testing a fresh specimen obtained at least 8 weeks later. Repeatedly reactive specimens that have a valid neutralization test, but are not neutralized by >50% are considered negative for neutralization by the criteria of the \square HIVAG-1 Monoclonal BLOCKING ANTIBODY assay. Specimens with a negative neutralization test result should be interpreted as indeterminate for HIV-1 p24 antigen. The interpretation of results of specimens found to be repeatedly reactive in the \square HIVAG-1 Monoclonal and negative in the additional, more specific 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 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 antigen and antibody.

LIMITATIONS OF THE PROCEDURE

The \square HIVAG-1 Monoclonal EIA detects uncomplexed HIV-1 p24 antigen in blood and thus is useful in screening blood and plasma donated for transfusion and further manufacture. The \square HIVAG-1 Monoclonal and \square HIVAG-1 Monoclonal BLOCKING ANTIBODY procedures and interpretation of results must be closely followed when testing serum or plasma specimens for the presence of HIV-1 p24 antigen. Because the procedures were designed to test individual serum or plasma specimens, insufficient data are available to interpret tests performed on other body fluid specimens, pooled blood or processed plasma or products made from such materials. Testing of these specimens is not recommended.

HIV-1 p24 antigen is present only transiently prior to seroconversion and later can be complexed by specific antibodies. HIV-1 p24 antigen testing should not be used in lieu of HIV-1 antibody testing as a screen for HIV-1 infection. A test result that is negative does not exclude the possibility of exposure to or infection with HIV-1. Negative results in this assay in individuals with prior exposure to HIV may be due to HIV-1 p24 antigen levels below the limit of detection of this assay. Resolution of the true status of HIV-1 infection may be obtained by testing a fresh blood specimen collected at least 8 weeks later for HIV-1 antibody.

The predictive value of a positive test is strongly influenced by the prevalence of HIV-1 infection in the population tested. For example, in low prevalence populations (Table II) the predictive value was 11.1% (1/9) while in populations with known HIV-1 infection (Table V), the predictive value was 97.1% (395/407). Therefore, the \square HIVAG-1 Monoclonal BLOCKING ANTIBODY test must be performed on all repeatedly reactive specimens.

AIDS and AIDS-related conditions are clinical syndromes and their diagnosis can only be established clinically. \square HIVAG-1 Monoclonal and \square HIVAG-1 Monoclonal BLOCKING ANTIBODY results should be used as an adjunct to other information available to the physician. \square HIVAG-1 Monoclonal testing alone cannot be used to diagnose AIDS even if the recommended investigation of reactive specimens indicates that HIV-1 p24 antigen is present.

SPECIFIC PERFORMANCE CHARACTERISTICS

1. PRECISION

Assay reproducibility for serum and plasma was determined by testing seven samples of which five were serum and two were plasma. Samples 1, 3, and 5 were prepared from serum obtained from three different antigen positive donors. Sample 6 was a dilution of Sample 5. Sample 7 was prepared from serum obtained from a pool of antigen negative donors. Samples 2 and 4 were prepared from two different HIV-1 antigen positive plasmapheresis donors. One site, Abbott Laboratories, tested the serum samples. Each serum sample was tested in replicates of five over four runs by three individuals using six instruments and one lot of \square HIVAG-1 Monoclonal. Three clinical sites tested the plasma samples. The plasma samples were randomized and repeated four times and each sample was tested in replicates of five using three lots of \square HIVAG-1 Monoclonal.

Intra-assay variability is based on an analysis of assay runs and inter-assay variability is based on an analysis of variability from all sources (Table I).

Samples	Number of Replicates	Mean S/CO	Intra-Assay SD	%CV	Total SD	Total %CV	
Serum	1	360	4.32	0.291	6.7	0.944	21.9
	3	360	3.07	0.268	8.7	0.583	19.0
	5	360	1.99	0.188	9.5	0.364	18.3
	6	360	1.45	0.245	16.9	0.383	26.4
	7	360	0.49	0.180	36.7	0.268	54.6
Plasma	2	180	4.12	0.268	6.5	0.881	21.4
	4	180	2.75	0.180	5.8	0.320	11.6

2. SPECIFICITY

A total of 10,270 serum and plasma specimens from random volunteer whole blood donors and plasmapheresis donors was collected from five geographically distinct U.S. blood centers (Table II). A study was conducted during a four-week period using randomly collected samples tested within 48 hours of collection. Specimens found to be repeatedly reactive were tested further with the \square HIVAG-1 Monoclonal BLOCKING ANTIBODY test within seven days of collection. All specimens were stored at 2-8°C between collection and testing. Two sites tested a total of 3,555 serum specimens with initially and repeatedly reactive rates of 0.42% and 0.0%, respectively. Two sites tested a total of 3,698 plasma specimens with initially and repeatedly reactive rates of 0.84% and 0.22%, respectively. One site tested a total of 3,017 plasmapheresis donors with initially and repeatedly reactive rates of 0.20% and 0.03%, respectively. Among the nine repeatedly reactive specimens, seven

specimens had a negative \square HIVAG-1 Monoclonal BLOCKING ANTIBODY result, one specimen did not have a valid \square HIVAG-1 Monoclonal BLOCKING ANTIBODY result, i.e. the mean signal produced by the specimen in the presence of Control Reagent (Solution B) was not greater than the cutoff value, and one specimen was positive for HIV-1 p24 antigen. The specimen that was positive for p24 antigen, tested negative for antibodies to HIV-1/2. A follow-up specimen obtained from this donor 11 days later was positive for antibodies to HIV-1/2 (see donor PD001 in Table VI).

When the HIV-1 p24 antigen positive donor found to be infected with HIV-1 on follow-up testing is removed from the calculation, the specificity of \square HIVAG-1TM Monoclonal was 99.92% (10,261/10,269, 99.85 to 99.97%, 95% binomial confidence interval) based on an assumed zero prevalence of HIV-1 p24 antigen in volunteer whole blood and plasma donors.

TABLE II
REACTIVITY IN LOW RISK POPULATIONS

Category	Number of Specimens	\square HIVAG-1 Monoclonal BLOCKING ANTIBODY		\square HIVAG-1 Monoclonal BLOCKING ANTIBODY Neutralization Results*		
		Initially Reactive (%)	Repeatedly Reactive (%)	N	I	P
Volunteer Blood Donors						
Serum	3,555	15 (0.42)	0			
Plasma	3,698	31 (0.84)	8 (0.22)	7 ^a	1 ^a	0
Plasmapheresis Donors	3,017	6 (0.20)	1 (0.03)	0	0	1 ^b
Total Donors	10,270	52 (0.51)	9 (0.09)	7	1	1

* Neutralization test results are categorized as N=Negative, defined as a valid \square HIVAG-1 Monoclonal BLOCKING ANTIBODY test (Mean S/CO of Solution B equal to or greater than 1.0) and a % neutralization less than 50%, I=Indeterminate, defined as an invalid \square HIVAG-1 Monoclonal BLOCKING ANTIBODY test (Mean S/CO of solution B is <1.0), and P=Positive, defined as a valid Antibody Blocking test (Mean S/CO of Solution B equal to or greater than 1.0) and % neutralization greater than or equal to 50%.

^a Specimens were negative for antibodies to HIV-1/HIV-2. No follow-up testing was performed.

^b Specimens were negative for antibodies to HIV-1/HIV-2. Follow-up specimen obtained from this donor 11 days later tested positive for antibodies to HIV-1/HIV-2.

A total of 235 repository serum or plasma specimens from individuals with medical conditions other than HIV-1 infection, including viral infections, bacterial infections, parasitic diseases, autoimmune diseases and cancer, as well as specimens containing potentially interfering substances was tested at two sites (Table III). Of the three specimens that were initially reactive, one (ANA) was not repeatedly reactive on retest, two specimens (anti-HSV and anti-Toxoplasma) were not retested in the \square HIVAG-1 Monoclonal test, but when tested in the \square HIVAG-1 Monoclonal BLOCKING ANTIBODY test, they had a valid \square HIVAG-1 Blocking test (Mean S/CO of Solution B equal to or greater than 1.0), but did not neutralize \geq 50%.

TABLE III
REACTIVITY IN SPECIMENS FROM PATIENTS WITH MEDICAL CONDITIONS OTHER THAN HIV-1 INFECTION OR SPECIMENS CONTAINING POTENTIALLY INTERFERING SUBSTANCES

Category	Number of Specimens	\square HIVAG-1 Monoclonal BLOCKING ANTIBODY		\square HIVAG-1 Monoclonal BLOCKING ANTIBODY Neutralization Results		
		Initially Reactive	Repeatedly Reactive	N	I	P
ANA	10	1	0			
anti-HBc	5	0	0			
anti-CMV	5	0	0			
anti-EBV	10	0	0			
E. coli Infections	10	0	0			
HBSAg	10	0	0			
anti-HBs	10	0	0			
anti-HCV	10	0	0			
anti-HIV-2	10	0	0			
anti-HSV	10	1	NP ^a		1 ^b	
anti-HTLV-1	10	0	0			
Hypergammaglobulinemia	10	0	0			
Multiple Myeloma	15	0	0			
Multiparous Female	10	0	0			
Rheumatoid Factor	10	0	0			
anti-Rubella	10	0	0			
anti-Toxoplasma	10	1	NP ^a		1 ^b	
Animal Handlers	35	0	0			
Elevated Triglycerides	10	0	0			
Elevated Bilirubin	10	0	0			
Elevated Hemoglobin	10	0	0			
TOTAL	235	3	0	2	0	0

^a NP=Repeat testing not performed

^b Specimens were reactive in the presence of \square HIVAG-1 Monoclonal BLOCKING ANTIBODY test Control Reagent, i.e. they had a valid neutralization test with S/CO equal to or greater than 1.0, but the specimen did not neutralize \geq 50% in the presence of blocking antibody. No follow-up testing was performed. Specimens had a low S/CO in the presence of Blocking antibody Control Reagent (Mean S/CO range 1.15-1.84).

Prospectively collected or repository serum or plasma specimens from 421 individuals with known risk factors for HIV-1 infection were tested at two sites. HIV-1 antibody status was unknown for these individuals (Table IV). There were 17 specimens that were initially reactive and seven that were \square HIVAG-1 Monoclonal repeatedly reactive for HIV-1 p24 antigen. Among the seven specimens that were repeatedly reactive, three specimens had a positive \square HIVAG-1 Monoclonal BLOCKING ANTIBODY neutralization test result, three specimens had indeterminate neutralization test results with invalid neutralization tests, and one specimen had a negative neutralization test result based on a valid neutralization test which did not neutralize \geq 50%. No follow-up testing was done.

TABLE IV
REACTIVITY OF HIV-1 p24 ANTIGEN IN GROUPS AT RISK FOR HIV-1 INFECTION

Category	Number of Specimens	\square HIVAG-1 Monoclonal BLOCKING ANTIBODY		\square HIVAG-1 Monoclonal BLOCKING ANTIBODY Neutralization Results		
		Initially Reactive	Repeatedly Reactive	N	I	P
Homosexual Men	50	0	0			
IVDUs ^a	150	3	0			
STD Patients ^b	50	4	1		1 ^c	
Hemophilia Patients	49	0	0			
Partners ^d of HIV-1 Infected Individuals	50	6	3		2 ^d	1 ^d
Pregnant Women at Risk for HIV-1 Infection	23	1	1			1 ^d
Prostitutes ^e	49	3	2		1 ^{d,e}	1 ^{d,e}
TOTAL	421	17	7	1	3	3

^a Gender unknown

^b 3 women, 47 men

^c 23 women, 26 men

^d Specimens were identified as HIV-1 antibody positive.

^e Specimens sourced from women

^f Specimens had low S/CO in the \square HIVAG-1 Monoclonal EIA (Mean S/CO range 1.29-1.66). These specimens were stored at 2-8°C for more than four days prior to testing in the \square HIVAG-1 Monoclonal BLOCKING ANTIBODY neutralization test. Studies of specimen storage conditions have shown that the HIV-1 p24 antigen signal produced in EIA and neutralization tests decline for some clinical specimens stored at 2-8°C. This effect was attributed to instability of HIV-1 p24 antigen when specimens were stored at 2-8°C.

3. SENSITIVITY

The sensitivity of \square HIVAG-1 Monoclonal was assessed by testing 1,592 repository serum specimens from HIV-1 seropositive individuals classified by CDC criteria for clinical stage of HIV-1 infection (Table V). The specimens were tested in a blinded study at five clinical sites. Of these 1,592 specimens, 415 (26.1%) were initially reactive, 407 (25.6%) were repeatedly reactive and 395 (97.1%) were positive for HIV-1 p24 antigen in the \square HIVAG-1 Monoclonal BLOCKING ANTIBODY neutralization test. Among the 407 repeatedly reactive specimens, seven specimens did not produce a valid signal in the neutralization test, i.e. the signal produced in the presence of Solution B was <1.0. All seven of these repeatedly reactive specimens had low EIA S/CO (Mean S/CO range 1.01-1.54) and were stored at 2-8°C for four days or more prior to testing in the \square HIVAG-1 Monoclonal BLOCKING ANTIBODY neutralization test. Studies suggest that the HIV-1 antigen signal produced by some clinical specimens declines over time when specimens are stored at 2-8°C. This effect is attributed to instability of antigen. Among the 400 clinical specimens that had a valid neutralization test, five specimens did not neutralize \geq 50%. Three of the five specimens produced a low S/CO (Mean S/CO range 1.33-2.21) and two had Mean S/CO's of 3.50 and 4.31.

Based on the positive neutralization test results, the prevalence of HIV-1 p24 antigen was estimated to be 9.7% in Asymptomatic patients, 34.8% in ARC patients, and 39.5% in AIDS patients. These results are comparable to previous estimates for HIV-1 antigen in clinical stages using a previously licensed assay.

TABLE V
HIV-1 p24 ANTIGEN SEROPREVALENCE IN HIV-1 ANTIBODY POSITIVE SUBJECTS STRATIFIED BY CLINICAL STAGE

Clinical Stage	Number of Specimens	\square HIVAG-1 Monoclonal BLOCKING ANTIBODY		\square HIVAG-1 Monoclonal BLOCKING ANTIBODY Neutralization Results			Prevalence %
		Initially Reactive (%)	Repeatedly Reactive (%)	N	I	P	
Asymptomatic	590	66 (11.2%)	62 (10.5%)	4	1	57	9.7%
ARC ^a	540	192 (35.6%)	191 (35.4%)	0	3	188	34.8%
AIDS	362	149 (41.2%)	147 (40.6%)	1	3	143	39.5%
Unknown Clinical Status	100	8 (8.0%)	7 (7.0%)	0	0	7	7.0%
TOTAL	1,592	415 (26.1%)	407 (25.6%)	5^b	7^c	395	24.8%

^a Includes Symptomatics classified by revised CDC criteria.TM

^b Specimens had a valid neutralization test, i.e. the signal produced in the presence of Blocking Antibody Control Reagent was \geq 1.0, but the specimen did not neutralize \geq 50% in the presence of Blocking Antibody. Three of five specimens had low S/CO in the \square HIVAG-1 Monoclonal EIA (Mean S/CO range 1.01-2.21). Two of the five specimens had a Mean S/CO of 3.50 and 4.31. Specimens were stored at 2-8°C for more than four days prior to testing with the \square HIVAG-1 Monoclonal BLOCKING ANTIBODY neutralization test.

^c Specimens did not have a valid \square HIVAG-1 Monoclonal BLOCKING ANTIBODY neutralization test, i.e. the signal produced in the presence of Blocking Antibody Control Reagent was <1.0. Specimens had a low S/CO (Mean S/CO range 1.01-1.54). Studies of specimen storage conditions have shown that the antigen signal declined for some clinical specimens stored at 2-8°C. This effect was attributed to instability of HIV-1 p24 antigen when specimens were stored at 2-8°C.

The ability of \square HIVAG-1 Monoclonal to detect HIV-1 p24 antigen was evaluated using eight commercially available seroconversion panels obtained from plasmapheresis donors (Table VI). In addition the test results for serial bleeds from the initially HIV-1 antigen positive/antibody negative plasmapheresis donor (PD001) described in Table II are included in Table VI. Results showed that \square HIVAG-1 Monoclonal detected HIV-1 p24 antigen prior to the appearance of detectable HIV-1 antibodies by approximately 11 days in Panel PD001, 10 days in Panel SV021, 7 days in Panels SV061, SV111, and SV161, and by 6 days in Panel SV091. \square HIVAG-1 Monoclonal detected HIV-1 p24 antigen concurrently with detection of antibody in Panels SV031 and SV071 and p24 antigen was not detected in Panel SV081.

The performance of \square HIVAG-1 Monoclonal test was evaluated by independent investigators using 31 seroconversion panels. Seroconversion was determined using licensed antibody screening tests (\square HIVAB HIV-1/HIV-2 (rDNA) EIA and Genetic Systems HIV-1/2 EIA). Panel specimens were also tested by the previously licensed HIV-1 antigen test, \square HIVAG-1, and two investigational HIV-1 p24 antigen tests. Both the \square HIVAG-1 Monoclonal and previously licensed \square HIVAG-1 detected HIV-1 antigen prior to seroconversion in 25 of 31 panels (80.6%). The \square HIVAG-1 Monoclonal EIA detected HIV-1 p24 prior to antigen being detected by the previously licensed polyclonal \square HIVAG-1 test. None of the HIV-1 antigen tests detected antigen in two of the panels tested.

TABLE VI
HIV-1 p24 ANTIGEN DETECTION IN SEROCONVERSION PANELS

Panel	Panel Member	Day of Donation	HIVAB HIV-1/ψV-2 (rDNA) EIA	HIVAG-1 Monoclonal S/C O	% Neutralization*	Antigen Status
SV021	A	1	—	2.545	100.1	+
	C	11	+	3.818	99.6	+
	D	18	+	0.610	—	—
SV031	A	1	—	0.403	—	—
	B	5	—	0.481	—	—
	C	8	—	0.429	—	—
	D	13	—	0.390	—	—
	E	20	—	0.377	—	—
	F	27	—	0.416	—	—
	G	29	—	0.416	—	—
	H	47	+	19.416	99.0	+
	I	49	+	25.974	99.2	+
	J	54	+	25.974	98.7	+
	K	55	+	25.974	98.2	+
SV061	A	1	—	3.545	100.3	+
	B	3	—	4.052	101.1	+
	C	8	+	14.935	99.5	+
	D	10	+	18.558	99.2	+
	E	16	+	9.208	99.2	+
SV071	A	1	—	0.455	—	—
	B	3	—	0.532	—	—
	C	17	+	3.026	100.6	+
	D	22	+	0.610	—	—
	E	28	+	0.429	—	—
SV081	A	1	—	0.429	—	—
	B	6	—	0.481	—	—
	C	23	—	0.532	—	—
	D	48	+	0.506	—	—
	E	61	+	0.390	—	—
SV091	A	1	—	19.792	99.4	+
	B	3	—	25.974	92.9	+
	C	7	+	13.013	99.6	+
	D	10	+	2.532	99.8	+
	E	17	+	0.844	—	—
SV111	A	1	—	25.974	99.3	+
	B	2	—	25.974	99.2	+
	C	6	—	25.974	99.7	+
	D	16	+	2.273	98.1	+
	E	20	+	1.532	100.8	+
SV151	A	1	—	1.299	97.1	+
	B	5	—	0.974	—	—
	C	8	—	2.091	102.3	+
	D	12	+	5.416	99.7	+
	E	16	+	12.610	99.6	+
PD001**	A	1	—	21.468	98.7	+
	B	9	—	0.766	—	—
	C	11	—	0.571	—	—
	D	22	+	0.324	—	—
	E	37	+	0.343	—	—

* Specimens with an HIVAG-1 Monoclonal EIA S/C O less than 1.0 were not tested using HIVAG-1 Monoclonal BLOCKING ANTIBODY test.
** Plasmaphoresis donor from donor population described in Table II.

The relative sensitivity of HIVAG-1 Monoclonal for HIV-1 p24 antigen and the previously licensed assay, HIVAG-1, were evaluated using the HIV-1 antibody positive specimens described in Table V. Both assays were repeatedly reactive for HIV-1 p24 antigen in 20.3% (323/1,592) of the specimens (Table VII). Following neutralization, 321 of the 323 specimens were positive by either HIVAG-1 Monoclonal or HIVAG-1 neutralization assays. Of these 321 specimens, 311 were positive by both assays, eight specimens were positive by HIVAG-1 Monoclonal and indeterminate (invalid) by HIVAG-1, one specimen was positive by HIVAG-1 Monoclonal and negative by HIVAG-1, and one specimen was indeterminate (invalid) by HIVAG-1 Monoclonal and positive by HIVAG-1.

Four of the 1,592 specimens (0.25%) were repeatedly reactive with HIVAG-1 only. Three of the four specimens were positive and one was negative following neutralization. A total of 84 specimens (5.28%) was repeatedly reactive with HIVAG-1 Monoclonal only. Following neutralization, 75 specimens were positive, four were negative, and five were indeterminate (invalid).

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 HIVAG-1 Monoclonal was 99.0% (395/399) for this set of specimens compared with a sensitivity of the previously licensed HIVAG-1 of 79.0% (315/399).

TABLE VII
HIV-1 p24 ANTIGEN DETECTION IN SPECIMENS TESTED BY HIVAG-1 MONOCLONAL AND HIVAG-1

HIV-1 Antigen Assay	Initial Assay		Repeat Assay		Neutralization Results*		P
	Nonreactive	Reactive	Nonreactive	Reactive	N	I	
HIVAG-1 Monoclonal and HIVAG-1	1165	329	1181	323	1	1	311**
HIVAG-1 only	86	12	84	4	1	0	3
HIVAG-1 Monoclonal only	12	86	4	84	4	5	75

* Neutralization test results are categorized as N=Negative, defined as a valid Antibody Blocking Test (Mean S/C O of Solution B equal to or greater than 1.0) and a % neutralization less than 50%; I=Indeterminate, defined as an invalid Antibody Blocking Test (Mean S/C O of solution B is less than 1.0); and P=Positive, defined as a valid Antibody Blocking Test (Mean S/C O of Solution B equal to or greater than 1.0) and % neutralization greater than or equal to 50%.
** 321 specimens were positive by either HIVAG-1 Monoclonal or HIVAG-1 neutralization assays. Of these 321 specimens, 311 were positive by both assays.

b Positive by HIVAG-1 Monoclonal and indeterminate by HIVAG-1.

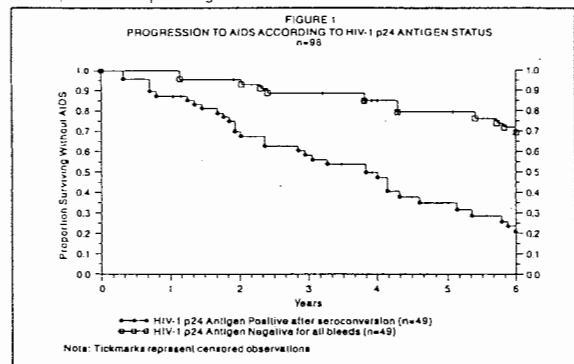
c Positive by HIVAG-1 Monoclonal and negative by HIVAG-1.

d Positive by HIVAG-1 and indeterminate by HIVAG-1 Monoclonal

4. HIV-1 p24 ANTIGEN AS A PROGNOSTIC MARKER

The value of HIV-1 p24 antigen positivity as a prognostic marker of progression to AIDS was evaluated with HIVAG-1 Monoclonal by testing yearly samples obtained longitudinally from 101 homosexual men enrolled in a prospective cohort study.^{59,60} Fifty-seven men were seropositive at entry into the study in 1982 and 44 men seroconverted during follow-up. One of the 57 seropositive subjects had no precise date of AIDS diagnosis and was not included in the analysis. The remaining 100 subjects were followed-up for a median of 7.0 years (range 1.1-13.1 years).

The cumulative incidence rates of AIDS according to HIV-1 p24 antigen status were calculated with the Kaplan-Meier method⁶¹ for 98 of the 100 subjects (Figure 1). Two seropositive subjects were removed from this analysis because antigenemia occurred after the onset of AIDS. The HIV-1 p24 antigen positive group (n=49) was comprised of all subjects with at least one antigen positive bleed after seroconversion and before the onset of AIDS. Year 0 for this group was defined as the time of the first p24 antigen positive bleed after seroconversion. The HIV-1 p24 antigen negative group (n=49) was comprised of those subjects who were negative for p24 antigen for all bleeds after seroconversion. Year 0 for this group was defined as the time of the first available bleed on or after seroconversion. There was a significant difference in rates of progression to AIDS between HIV-1 p24 antigen positive and negative subjects (p=0.0013 at two years, p=0.0001 at four years, and p=0.0001 at six years using the Log-rank test). From the Kaplan-Meier analysis of the cohort of 98 homosexual men censored at six years, a regression analysis of survival data based on the Cox's proportional hazards model (p=0.0001, Wald chi-squared test)⁶² was performed in order to calculate the relative risk of an HIV-1 infected individual with or without detectable HIV-1 p24 antigen developing AIDS. This relative risk indicates that over a six year time period, an HIV-1 infected individual with detectable HIV-1 p24 antigen is 4.489 times more likely to develop AIDS than an individual with no detectable HIV-1 p24 antigen.



Over the follow-up period, progression to AIDS according to HIV-1 p24 antigen status could be determined among cohort subjects (Table VIII). Of the 49 HIV-1 p24 antigen positives, 34 (69.4%) had developed AIDS a median of 2.5 years from Year 0 when censored at six years. Of the 49 subjects who were negative for HIV-1 p24 antigen, 13 (26.5%) developed AIDS a median of 4.3 years from Year 0 when censored at six years. These results illustrate the prognostic value of this test.

TABLE VIII
PROGRESSION TO AIDS ACCORDING TO HIV-1 p24 ANTIGEN STATUS

p24 Antigen Status	Number of Subjects	Study Censored at Six Years	
		Number With AIDS	Median Time of AIDS (years)*
Positive	49	34 (69.4%)	2.5
Negative	49	13 (26.5%)	4.3

* Median time to AIDS diagnosis is presented relative to Year 0.

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