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# ANTIBODY TO HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HUMAN) HIV-1 p24 Antigen Neutralization Kit

## NAME AND INTENDED USE

HIV-1 p24 Antigen Neutralization Kit consists of a set of reagents and instructions intended to be used only with HIV-1 p24 Antigen ELISA Test System (available separately) as a qualitative, *in vitro* test for the presence of p24 antigen of the Human Immunodeficiency Virus Type 1 (HIV-1) in human plasma or serum. The neutralization test is intended to be used as an additional, more specific test for specimens found to be repeatedly reactive in HIV-1 p24 Antigen ELISA Test System and as an aid in the diagnosis of HIV-1 infection and prognosis or monitoring of disease progression.

## SUMMARY AND EXPLANATION OF THE TEST

HIV-1 viral infection in humans is characterized by periods of antigenemia in which HIV-1 antigens are detectable in blood<sup>1-3,4,5</sup>. One of the viral antigens present in blood during antigenemia is the core protein, p24, the major internal structural protein of HIV-1. HIV-1 p24 Antigen ELISA Test System is an enzyme-linked immunosorbent assay for the detection of the human HIV-1 p24 core protein using a monoclonal capture antibody specific for HIV-1 p24 antigen. The HIV-1 p24 Antigen Neutralization Kit is intended as an additional, more specific test to detect the presence of HIV-1 p24 antigen in specimens found to be repeatedly reactive in HIV-1 p24 Antigen ELISA Test System.

## BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The reagents and instructions provided in HIV-1 p24 Antigen Neutralization Kit are to be used only with the materials supplied with HIV-1 p24 Antigen ELISA Test System (available separately) as a test for the presence of HIV-1 p24 antigen in human serum or plasma. The neutralization test is a qualitative assay that uses the principle of specific antibody-antigen complex formation to reduce the amount of free HIV-1 p24 antigen available for reacting in the HIV-1 p24 Antigen ELISA Test System. Purified human anti-HIV-1 IgG (Neutralization Reagent) is placed in a microwell which is coated with HIV-1 p24 antigen-specific monoclonal antibody. A specimen is then placed in the microwell and incubated. HIV-1 p24 antigens in the specimen, if present, form complexes with the Neutralizing Reagent, and the amount of antigen available to bind to the antibody-coated microwell is reduced. For comparison, normal human globulins (Negative Neutralizing Control) is added to another microwell and the same specimen is added and incubated. HIV-1 p24 antigens in a specimen, if present, are unaffected by the presence of Negative Neutralizing Control, and binding to the antibody-coated microwell proceeds unhindered. At this point, the remaining steps of the assay described in the package insert of HIV-1 p24 Antigen ELISA Test System are performed. Color development is directly proportional to the amount of free HIV-1 p24 antigen present in the microwells, and absorbance is measured using a spectrophotometer. If HIV-1 p24 antigen is present in a specimen, color development for that specimen tested in the presence of Neutralizing Reagent will be reduced by 40% or more when that specimen is tested in the presence of Negative Neutralizing Control.

## REAGENTS

HIV-1 p24 Antigen Neutralization Kit (Ortho Product Code 933185)

1 vial Neutralizing Reagent (NR) (Human) (1.0 mL)

Source: Human anti-HIV-1 IgG; nonreactive for hepatitis B surface antigen (HBsAg), antibodies to hepatitis C virus (HCV) and HIV-1 antigen(s)

Preservative: 0.1% sodium azide

1 vial Negative Neutralizing Control (NNC) (Human) (1.0 mL)

Source: Human Globulins, Reagent Grade, found nonreactive for hepatitis B surface antigen (HBsAg), antibodies to hepatitis C virus (HCV) and HIV-1 antigen(s)

Preservative: 0.1% sodium azide

## WARNINGS AND PRECAUTIONS

### For *In Vitro* Diagnostic Use

#### Safety Precautions

Handle all biological materials in HIV-1 p24 Antigen ELISA Test System and HIV-1 p24 Antigen Neutralization Kit as though capable of transmitting infectious agents. The Biotin Reagent, NHS and HIV-1 p24 Antigen Reagent have been inactivated. No known test method can offer complete assurance that products derived from human blood will not transmit infection.

Handle all biological specimens as though capable of transmitting infection. We recommend that these reagents, waste from plate washers and blood specimens be handled according to established Good Laboratory Practices (GLP), Occupational Safety and Health Administration (OSHA) and the Centers for Disease Control and Prevention (CDC) guidelines in the United States of America, and in all other countries by following the equivalent of the GLP, OSHA and CDC recommendations.

1. Wear disposable gloves while handling potentially infectious specimens and kit reagents. Upon completion, remove gloves and wash hands thoroughly.
2. Spills involving non-acidic liquids should be wiped promptly and thoroughly with a 0.5% solution of sodium hypochlorite, or other effective disinfectant to decontaminate the area.<sup>6-7</sup> Spills involving acidic liquids should be wiped dry first and then the area should be wiped with a 0.5% solution of sodium hypochlorite or equivalent disinfectant to decontaminate. Materials used to wipe up spills should be treated as hazardous waste.
3. Do not pipette by mouth.
4. Do not smoke, eat or drink in areas where specimens or kit reagents are handled.
5. Some of the reagents in this product contain sodium azide. Sodium azide under acidic conditions yields hydrazoic acid, an extremely toxic compound. After contamination, 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 accumulation of deposits in metal piping in which explosive conditions could develop.
6. Do not pour bleach and 4N Sulfuric Acid (H<sub>2</sub>SO<sub>4</sub>) for Ortho ELISA Test System into sink at the same time.
7. Dispose of all materials that have come into contact with specimens and reagents in accordance with local, state and federal regulations<sup>8</sup>. 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 his decontamination cycle using biological indicators.<sup>9</sup>

#### Handling Precautions

1. Do not use reagents beyond their labeled expiration date.

2. Do not mix reagents from kits with different lot numbers. Any lot of Wash Buffer (20X Concentrate) and 4N Sulfuric Acid ( $H_2SO_4$ ) for Ortho ELISA Test System may be used provided it is not beyond its labeled expiration date.
3. Avoid microbial contamination of specimens, reagents and water used for reconstitution of reagents and preparation of Wash Buffer. Avoid chemical contamination of reagents and equipment.
4. Distilled or deionized water must be used for reconstitution of reagents and preparation of Wash Buffer. Clinical laboratory reagent water Type I or Type II is acceptable<sup>19</sup>. Store water in nonmetallic containers.
5. Cross-contamination between reagents will invalidate the test results. Labeled, dedicated reservoirs for the appropriate reagents are recommended.
6. The microwell strips are sealed in protective pouches with a humidity indicator. The desiccant, normally blue/purple in color, will turn pink if moisture is present in the pouch. If the desiccant is pink, the microwell strips should not be used.
7. Ensure the specimen is added to the microwell. Failure to add specimen may produce an erroneous result.
8. When using a single-channel micropipette for manual sample addition, use a new pipette tip for each specimen to be assayed. When using a multichannel micropipette, new tips are to be used for each reagent to be added.
9. Strict adherence to the specified wash procedure is crucial to ensure optimum assay performance (See Step 7 of Test Procedure).
10. Do not allow microwells to become dry once the assay has begun.
11. Do not touch the bottom exterior surface of the microwells. Fingerprints or scratches may interfere with reading the microwells.
12. Ensure that the microwell strips are level in the microwell strip holder during the test procedure. If necessary, wipe the bottom of the microwell strips carefully with a soft, lint-free, absorbent tissue to remove any moisture before reading.
13. Do not allow sodium hypochlorite fumes from chlorine bleach or other sources to contact the microwell strips during the assay, as the reaction may be inhibited.
14. All pipetting equipment should be used with care, calibrated regularly and maintained following equipment manufacturer's instructions.
15. The microwell reader should contain a 570 nm reference filter. If an instrument without a reference filter is used, areas in the bottom of the microwells that are opaque, scratched or irregular may cause elevated readings.
16. HIV-1 p24 Antigen Neutralization Kit (Ortho Product Code 933185) should be used in conjunction HIV-1 p24 Antigen ELISA Test System (Ortho Product Codes 933184 and/or 933180).

#### PREPARATION OF REAGENTS

Neutralization Reagent and Negative Neutralizing Control require no further dilution prior to use. See package insert for preparation of reagents supplied with HIV-1 p24 Antigen ELISA Test System.

#### STORAGE INSTRUCTIONS

1. Neutralization Reagent and Negative Neutralizing Control should be stored at 2-8°C.
2. When not in use, store the test kit at 2-8°C. Do not freeze the test kit.
3. Store reconstituted reagents at 2-8°C.
4. Bring reagents to room temperature before use and return to storage conditions indicated immediately after use.

#### INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

Alteration in the physical appearance of the test kit may indicate instability or deterioration. Runs which do not meet the negative and positive control acceptance criteria in the QUALITY CONTROL PROCEDURES section may indicate technique error or deterioration of the kit reagents or TMB Substrate Solution. Such runs must be repeated.

#### SPECIMEN COLLECTION AND PREPARATION

1. The specimens to be tested with HIV-1 p24 Antigen Neutralization Kit are those found to be repeatedly reactive with HIV-1 p24 Antigen ELISA Test System.
2. Plasma collected in acid-citrate-dextrose (ACD), citrate-phosphate-dextrose with adenine (CPDA-1), EDTA, sodium citrate and 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. Blood should be collected by approved medical techniques. Plasma collected with an improper ratio of specimen to anticoagulant should not be used. Remove the serum from the clot and plasma from the red cells as soon as possible to avoid hemolysis. HIV-1 p24 Antigen ELISA Test System is not affected by elevated hemoglobin up to 350 mg/dL or triglycerides up to 474 mg/dL.
3. Specimens that produce an absorbance value less than 2.000 in the repeat tests of HIV-1 p24 Antigen ELISA Test System should be used without dilution in HIV-1 p24 Antigen Neutralization Kit. Specimens with absorbance values greater than or equal to 2.000 should be diluted at least 1:2 or until the absorbance value of the specimen falls below 2.000 when tested with HIV-1 p24 Antigen Neutralization Kit. Use the Normal Human Serum (NHS) reagent supplied in HIV-1 p24 Antigen ELISA Test System to dilute the specimen.
4. 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 longer storage is necessary, the specimens should be frozen at -20°C or below for up to three years. Storage of specimens in self-defrosting freezers is not recommended. Specimens should be mixed thoroughly after thawing and brought to room temperature prior to testing. Avoid subjecting specimens to

repeated 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.

5. Do not use heat-inactivated specimens.
6. Performance has not been established using cadaveric specimens or body fluids other than serum or plasma, such as urine, saliva or pleural fluid.
7. Bring all specimens to room temperature (15-30°C) prior to assay.
8. Do not use azide to preserve specimens. Do not test patient or donor specimens containing azide. Sodium azide inhibits peroxidase activity.
9. Specimens containing precipitates may give erroneous results and should be clarified prior to assaying.
10. 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

HIV-1 p24 Antigen Neutralization Kit  
50 Test Kit (Ortho Product Code 933185)  
(See REAGENTS for complete listing)

### Materials Required But Not Provided

1. Wash Buffer (20X Concentrate) is sold separately (Ortho Product Code 933186, 4 x 1.9 Liters).
2. 4N Sulfuric Acid (H<sub>2</sub>SO<sub>4</sub>) for Ortho ELISA Test System is sold separately (available in the United States from Ortho Diagnostic Systems Inc., Product Code 933040). Any lot number of 4N Sulfuric Acid (H<sub>2</sub>SO<sub>4</sub>) for Ortho ELISA Test System may be used provided it is not used beyond its labeled expiration date.
3. HIV-1 p24 Antigen ELISA Test System (Ortho Product Code 933184 and/or 933180).
4. Adjustable multichannel micropipette capable of delivering 50µL and 200µL with at least ± 5% accuracy or equivalent reagent dispenser.
5. Fixed or adjustable single-channel micropipettes capable of delivering 10- 200µL and 200-1000µL with at least ± 5% accuracy or equivalent reagent dispenser.
6. Disposable pipette tips or equivalent.
7. Appropriately sized serological pipette or graduated cylinder.
8. Multichannel micropipette reservoir or equivalent reagent container.
9. Multichannel aspirator-washer device capable of dispensing and aspirating at least 400µL per well (Consult the device's operator's manual for additional technical information).
10. Dual wavelength microwell reader capable of reading at 450 nm with a reference filter of 570 nm. If an instrument without a reference filter is used, areas in the bottom of the microwells that are opaque, scratched or irregular may cause elevated readings. Linearity of the microwell reader must range from at least 0 to 2.5 absorbance units. Consult the instrument manufacturer's specifications.
11. 37°C ± 2°C incubator (dry or humidified).
12. Distilled or deionized water, clinical laboratory reagent water Type I or Type II is acceptable. (See Handling Precautions section).
13. 5.25% sodium hypochlorite (chlorine bleach) or equivalent effective disinfectant.
14. White microwell strips (Ortho Product Code 936980) or equivalent uncoated microwells.
15. Variable speed microwell plate shaker capable of 100 to 400 rpm, optional.

### Test Procedure

1. Approximately 30 minutes prior to the beginning of the procedure, bring kit components to room temperature. Invert reagents gently several times, but avoid foaming. Check the incubator temperature; maintain at 37°C ± 2°C.
2. Determine the total number of wells needed for the assay.

		Number of Wells Required
Assay Control Strip	Substrate Blank	1
	Negative Control	3
	Positive Control	2
Control Strip (NNC)	Antigen Control	2
	Test Specimen	1 each
Test Strip (NR)	Antigen Control	2
	Test Specimen	1 each

See Figure 1 for Suggested Plate Diagram.

Figure 1. Recommended Plate Configuration for HIV-1 p24 Antigen Neutralization Kit

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK	ANTIGEN CONTROL	SPECIMEN 7		ANTIGEN CONTROL	SPECIMEN 7						
B	NEG CONTROL	ANTIGEN CONTROL	SPECIMEN 8		ANTIGEN CONTROL	SPECIMEN 8						
C	NEG CONTROL	SPECIMEN 1	SPECIMEN 9		SPECIMEN 1	SPECIMEN 9						
D	NEG CONTROL	SPECIMEN 2	SPECIMEN 10		SPECIMEN 2	SPECIMEN 10						
E	POS CONTROL	SPECIMEN 3	SPECIMEN 11		SPECIMEN 3	SPECIMEN 11						
F	POS CONTROL	SPECIMEN 4	SPECIMEN 12		SPECIMEN 4	SPECIMEN 12						
G	Empty	SPECIMEN 5	SPECIMEN 13		SPECIMEN 5	SPECIMEN 13						
H	Empty	SPECIMEN 6	SPECIMEN 14		SPECIMEN 6	SPECIMEN 14						
	ASSAY CONTROL STRIP	CONTROL STRIP (NNC)	CONTROL STRIP (NNC)		TEST STRIP (NR)	TEST STRIP (NR)						

NOTE: The term Control Strip (NNC) is used to define all strips where Negative Neutralizing Control (NNC) has been added to the microwells. The term Test Strip (NR) is used to define all strips where Neutralizing Reagent (NR) has been added to the microwells.

If the entire microwell plate is not required, an appropriate number of microwell strips can be assembled into a partial plate. Unused strips of wells should be stored at 2 - 8°C in the supplied foil pouch, with desiccant, tightly sealed and used within 60 days of opening the pouch. Record the date the pouch is opened and the expiration date of the unused wells on the foil pouch.

Performing the test on less than a full plate is permitted as long as the following conditions are met:

Microwell strips from different plates can be mixed to assemble full or partial plates as long as they are from the same lot, within the open pouch expiration date and have come from plates that have previously demonstrated proper response to kit controls.

When assembling a plate which contains strips from a newly opened, previously untested plate, these strips should be placed at the beginning of the plate and receive the full complement of kit controls.

**CAUTION: Handle the microwell strips with care. Do not touch the bottom exterior surface of the wells.**

3. It is suggested (but not required) that the Test Strips be separated from the Control Strips by a column of white or uncoated strips. Refer to Figure 1 for a suggested plate diagram.
4. Prepare a record (plate map) identifying the placement of controls and specimens in the Control and Test Strips.
5. Verify that any manual dispensing equipment is set to deliver the specified volumes stated in the procedure, following the equipment manufacturer's instructions. Add reagents, controls and specimens to the microwells in the order specified.
  - a. Add 20µL of Negative Neutralizing Control to all wells of the Control Strips except 1A, 1B, 1C, 1D, 1E and 1F. These microwells will contain the assay controls.
  - b. Add 20µL of Neutralizing Reagent to all wells of the Test Strips (NR).
  - c. Add 200µL of Negative Control (NHS) to wells 1B, 1C, and 1D of the Assay Control Strip.  
Note: These wells will serve as assay Negative Controls.
  - d. Add 200µL of NHS to wells 1E and 1F of the Assay Control Strip, then add 50µL of the reconstituted HIV-1 p24 Antigen Reagent to those two wells.  
Note: These wells will serve as assay Positive Controls.
  - e. Add 200µL of NHS to duplicate wells of the Control Strips (NNC) and Test Strips (NR), then add 50µL of the reconstituted HIV-1 p24 Antigen Reagent to these wells.  
Note: These wells will serve as Antigen Controls.
  - f. Add 200µL of each test specimen to one well of the Control Strip (NNC) and to one well of the Test Strip (NR).
  - g. Add 20µL of Lyse Buffer to all wells except 1A.
  - h. If the controls, specimens and Lyse Buffer have been manually delivered, assure that the microwells are thoroughly mixed. Use of a microwell plate shaker or manual mixing with a pipette are acceptable. The shaker should be used at a slow to moderate speed, taking care to avoid splashing of the contents of the test wells.

NOTE: If a microwell plate shaker is used for mixing, the plate sealer may be applied prior to shaking.

6. For manual processing of the microwell plates, cover the microwell strip holder with a plate sealer. When using an automated microplate processor for incubation, follow the manufacturer's recommendations with regard to microplate sealing. Incubate at 37°C ± 2°C for 60 minutes ± 5 minutes.

7. Level the strips in the microwell strip holder, if necessary. With an aspirator-washer device, aspirate and then wash all wells six times with Wash Buffer (1X).

**CAUTION: Strict adherence to the specified wash procedure is crucial to ensure optimum assay performance. Follow the steps specified in order to ensure thorough washing.**

- a. Aspirate the sample solutions from the microwells, then fill completely with Wash Buffer (1X). Do not allow wells to overflow. Allow approximately 25 seconds between the addition of Wash Buffer and subsequent aspiration.
  - b. Complete the aspirate/fill sequence five additional times.
  - c. Completely aspirate wells. Invert the plate and firmly tap on a clean paper towel to remove excess Wash Buffer, if necessary.
8. Add 200µL of Biotin Reagent to all wells except 1A.
  9. For manual processing of microwell plates, cover the microwell strip holder with a new plate sealer. When using an automated microplate processor for incubation, follow the manufacturer's recommendations with regard to microwell plate sealing. Incubate at 37°C ± 2°C for 60 minutes ± 5 minutes.
  10. After the second incubation, wash the wells as described in Step 7.
  11. Add 200µL of SA-HRPO Working Solution to all wells except 1A. Do not use more than a single preparation of SA-HRPO Working Solution on a plate.
  12. For manual processing of microwell plates, cover the microwell strip holder with a new plate sealer. When using an automated microplate processor for incubation, follow the manufacturer's recommendations with regard to plate sealing. Incubate at 37°C ± 2°C for 30 minutes ± 2 minutes.
  13. After the third incubation, wash the wells as described in Step 7.
  14. Add 200µL of TMB Substrate Solution to all wells, including 1A. Do not use more than a single preparation of TMB Substrate Solution on a plate.
  15. Incubate at room temperature in the dark for 30 minutes ± 2 minutes.
  16. Add 50µL of 4N Sulfuric Acid (H<sub>2</sub>SO<sub>4</sub>) for Ortho ELISA Test System to all wells, including 1A. To ensure proper mixing, acid should be added forcibly in a steady stream. If necessary, gently tap the plate or use a microwell plate shaker to mix the contents. Care should be taken to avoid splashing of the contents of the microwells. When using an automated microplate processor, follow the instrument manufacturer's instructions with regard to mixing.
  17. If necessary, wipe moisture from the bottom of the microwell strips with a soft, lint-free, absorbent tissue before reading. If necessary, level the strips in the microwell strip holder. Read the microwell plate at a wavelength of 450 nm. For dual wavelength readers set the reference wavelength at 570 nm. Blank the reader on well 1A according to the instrument manufacturer's instructions.

The user should ensure that the blank value (well 1A) has been subtracted from all control and specimen well values prior to applying the Quality Control criteria below.

**NOTE:** Microwell strip plates must be read within 30 minutes following the addition of 4N Sulfuric Acid (H<sub>2</sub>SO<sub>4</sub>) for Ortho ELISA Test System. Plates should be stored in the dark until read.

## QUALITY CONTROL PROCEDURES <sup>11,12</sup>

### 1. Substrate Blank Acceptance Criteria

A plate is considered valid if the absorbance value of the substrate blank (well 1A) is greater than or equal to 0.000 and less than or equal to 0.050.

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

### 2. Negative Control Acceptance Criteria

- a. Individual negative control values must be greater than or equal to 0.000 and less than or equal to 0.100. If one of the three negative control values is outside either of these limits, recalculate the negative control mean (NCx) based on the other two acceptable control values. The plate is invalid and the test must be repeated if two or more of the three controls values are outside these limits.

- b. Determine the mean negative control value (NCx)

Example:

<u>Negative Control</u>	<u>Absorbance</u>
1	0.058
2	0.046
3	0.058
Total Absorbance	= 0.162
NCx = $\frac{\text{Total Absorbance}}{3}$	= 0.054

### 3. Positive Control Acceptance Criteria

- a. Determine the mean positive control value (PCx)

Example:

<u>Positive Control</u>	<u>Absorbance</u>
1	1.000
2	1.215
Total Absorbance	= 2.215
PCx = $\frac{\text{Total Absorbance}}{2}$	= 1.108

- b. The expected range of the mean positive control value is greater than or equal to 0.600 and less than or equal to 1.600. The assay is invalid and the test must be repeated if the mean positive control value is outside either of these limits.

- c. Individual positive control absorbance values must be within  $\pm 25\%$  of the mean positive control value. The assay is invalid and the test must be repeated if either of the positive controls is outside these limits.  
 Example:  $0.25 \times 1.108 = 0.277$   
 Range = 0.831 to 1.385

In the above example, both of the Positive Control values are valid.

4. Calculation of the Cutoff Value

- a. Cutoff Value =  $NCx + 0.050$

Example:

<u>Negative Control</u>	<u>Absorbance</u>
1	0.058
2	0.046
3	<u>0.058</u>
Total Absorbance	= 0.162
$NCx = \frac{\text{Total Absorbance}}{3}$	= 0.054

$$\text{Cutoff Value} = 0.054 + 0.050 = 0.109$$

- b. The expected range of the Cutoff Value is greater than or equal to 0.050 and less than or equal to 0.150. If the Cutoff Value falls outside of this range, the assay should be repeated.

5. Antigen Control (Control Strip (NNC)) Acceptance Criteria

- a. Determine the mean Antigen Control absorbance value ( $AgCx$  (Control Strip (NNC))) by adding the absorbance values of the two wells containing the Antigen Control on the Control Strip (NNC) and dividing by 2.

Example:

<u>AgCx (Control Strip (NNC))</u>	<u>Absorbance</u>
1	1.002
2	<u>0.989</u>
Total Absorbance	1.991

$$AgCx \text{ (Control Strip (NNC))} = \frac{\text{Total Absorbance}}{2} = 0.996$$

- b. The expected range of the mean Antigen Control (Control Strip (NNC)) is greater than or equal to 0.600 and less than or equal to 1.600.

6. Antigen Control (Test Strip (NR)) Percent Reduction Acceptance Criteria

- a. Determine the mean Antigen Control absorbance value ( $AgCx$  (Test Strip (NR))) by adding the absorbance values of the two wells containing the Antigen Control on the Test Strip (NR) and dividing by 2.

Example:

<u>AgCx (Test Strip (NR))</u>	<u>Absorbance</u>
1	0.431
2	<u>0.300</u>
Total Absorbance	= 0.731

$$AgCx \text{ (Test Strip (NR))} = \frac{\text{Total Absorbance}}{2} = 0.366$$

- b. Calculate the percent reduction using the mean Negative Control Value (NCx) and the mean Antigen Control absorbance values ( $AgCx$ ) for both the Control Strip (NNC) and Test Strip (NR).

$$\text{Percent Reduction} = 1 - \frac{AgCx \text{ (Test Strip (NR))} - NCx}{AgCx \text{ (Control Strip (NNC))} - NCx} \times 100$$

Example:

$$\text{Percent Reduction} = 1 - \frac{(0.366 - 0.054)}{(0.996 - 0.054)} \times 100$$

$$1 - \frac{(0.312)}{(0.942)}$$

$$(1 - 0.331) \times 100$$

$$(0.669) \times 100$$

$$\text{Percent Reduction} = 66.9\%$$

- c. The calculated percent reduction for the Antigen Control must be greater than or equal to 40% or the assay is invalid and must be repeated.

7. Specimen Acceptance Criteria

- a. The absorbance value of the test specimen on the Control Strip (NNC) should be greater than or equal to the Cutoff Value and less than 2.000. If the absorbance value is less than the Cutoff Value, the specimen result is invalid and HIV-1 p24 Antigen Neutralization test should be repeated for that specimen using the original specimen or a fresh specimen.

If the absorbance value of the test specimen on the Control Strip (NNC) is greater than or equal to 2.000, the specimen should be diluted as described in the SPECIMEN COLLECTION AND PREPARATION section. The HIV-1 p24 Antigen Neutralization test should then be performed again for that specimen.

b. Calculate the percent reduction for the test specimen:

$$\text{Percent Reduction} = 1 - \frac{\text{Specimen (Test Strip (NR))} - \text{NCx}}{\text{Specimen (Control Strip (NNC))} - \text{NCx}} \times 100$$

Example:

$$\text{Percent Reduction} = 1 - \frac{(0.451) - (0.054)}{(1.259) - (0.054)} \times 100$$

$$1 - \frac{(0.397)}{(1.205)} \times 100$$

$$= (1 - 0.329) \times 100$$

$$= 0.671 \times 100$$

$$\text{Percent Reduction} = 67.1\%$$

## INTERPRETATION OF RESULTS

Control Strip (NNC) Absorbance Value	Percent Reduction	Results	Interpretation
≥ Cutoff Value and < 2.000	≥ 40%	Neutralized	Positive
≥ Cutoff Value and < 2.000	< 40%	Non-Neutralizing	Indeterminate
< Cutoff Value	NA	Invalid Neutralization Test	Indeterminate Retest Sample
≥ Cutoff Value and ≥ 2.000	NA	NA	Dilute and retest

- Specimens with absorbance values on the Control Strip (NNC) greater than or equal to the Cutoff Value and less than 2.000 (with or without dilution) where percent reduction on the Test Strip (NR) is greater than or equal to 40% are considered neutralized in the HIV-1 p24 Antigen Neutralization Test. These specimens are considered **positive** for HIV-1 p24 antigen. In the absence of antibody reactivity or other indications of HIV-1 infection, the possibility exists that HIV-1 p24 antigen reactivity precedes evidence of seroconversion. The true state of HIV-1 infection may be clarified and resolved by obtaining a fresh specimen at least 8 weeks later and testing for HIV-1 antigen and antibodies.
- Specimens with a percent reduction less than 40% in the neutralization test are considered negative for neutralization, i.e., non-neutralizing, but should be considered **indeterminate** for HIV-1 p24 antigen. The interpretation of results of test specimens found to be repeatedly reactive in HIV-1 p24 Antigen ELISA Test System and negative in the additional, more specific HIV-1 p24 Antigen Neutralization Test 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 or by retesting after at least 8 weeks for HIV-1 antibodies.
- Specimens that do not meet all of the criteria for a valid neutralization test are considered **indeterminate** for HIV-1 p24 antigen. An indeterminate neutralization test result may indicate deterioration of the test specimen or technical error. Clarification may be obtained by retesting the original specimen or testing a fresh specimen. Specimens that are repeatedly reactive in HIV-1 p24 Antigen ELISA Test System and indeterminate in HIV-1 p24 Antigen Neutralization Test must not be considered positive or negative and the original specimen should be tested again using HIV-1 p24 Antigen Neutralization test, or a fresh specimen should be tested according to the recommendations of the HIV-1 p24 Antigen ELISA Test System. Follow-up testing of a fresh specimen obtained after 8 weeks for HIV-1 antibodies should also be performed.

## LIMITATIONS OF THE PROCEDURE

One should closely follow the procedures and interpretation of results recommended in the HIV-1 p24 Antigen ELISA Test System package insert and the HIV-1 p24 Antigen Neutralization Kit package insert.

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

AIDS and AIDS-related conditions are syndromes that can only be established by clinical diagnosis. 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 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, presented in the Performance Characteristics section of this package insert and in the HIV-1 p24 Antigen ELISA Test System package insert, suggest that testing specimens in the HIV-1 p24 Antigen ELISA Test System and the HIV-1 p24 Antigen Neutralization Kit 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, which are known to vary with different stages of the disease, 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 the HIV-1 p24 Antigen ELISA Test System package insert for more information).

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 test procedure and the Interpretation of Results sections 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. Test results, either positive or negative, for an individual specimen should not be considered predictive of test results for specimens collected at a later time from the same person.

Data obtained from testing blood specimens of persons known to be infected and of 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.

#### PERFORMANCE CHARACTERISTICS OF THE NEUTRALIZATION TEST

The HIV-1 p24 Antigen Neutralization test is a qualitative *in vitro* test that is intended to be used as an additional, more specific test for the presence of HIV-1 p24 antigen in human plasma or serum specimens found to be repeatedly reactive in HIV-1 p24 Antigen ELISA Test System. Extensive clinical studies were conducted to evaluate the performance of HIV-1 p24 Antigen ELISA Test System and the HIV-1 p24 Antigen Neutralization Kit. Complete clinical performance data may be found in the package insert for HIV-1 p24 Antigen ELISA Test System. Data on the performance of the HIV-1 p24 Antigen Neutralization Kit are summarized below.

##### A. Performance with Repeatedly Reactive Specimens

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

Table 1. Performance of the 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>a</sup>	0	2 (0.00066) <sup>e</sup>
Asymptomatic (CDCII) <sup>d</sup>	505	108	1 <sup>f</sup>	10 <sup>f</sup>	97 (19.2)
ARC (CDCIII) <sup>d</sup>	364	182	3 <sup>f</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>b</sup>	358	154	0	0	154 (43.0)
CD <sub>4</sub> 201-500 <sup>b</sup>	144	81	0	0	81 (56.3)
CD <sub>4</sub> ≤ 200 <sup>b</sup>	67	42	0	0	42 (62.7)

- a AAB8 Central laboratory repeatedly reactive specimens, as described in Table 2
- 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 of the Antigen Assay.
- 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 of the HIV-1 p24 Antigen Assay.
- h Includes data from Table 10 the HIV-1 p24 Antigen Assay.

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 1, 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 over 6 days, 37% were tested over 7 days and 42% were tested ≥ 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 HIV-1 p24 Antigen Neutralization Kit 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, with initial assay OD values from 0.165 down to 0.069.

neutralization test 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 HIV-1 p24 Antigen ELISA Test System for HIV-1 p24 antigen.

**C. Reproducibility of the HIV-1 P24 Antigen Neutralization Kit**

The reproducibility of the HIV-1 p24 Antigen Neutralization test was determined with the coded panel of paired fresh and frozen specimens as part of the clinical study. Table 2 shows the test results based on 24 determinations for these panel members.

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

Panel Member	Condition	Mean Absorbance Specimen + NNC OD <sub>450/570 nm</sub>	Mean Absorbance Specimen + NR OD <sub>450/570 nm</sub>	% Signal Reduction <sup>a</sup>	Signal/CO	SD	%CV
1	Fresh	0.693	0.056	94.4	8.1	0.01	1.94
	Frozen	0.705	0.059	93.6	8.2	0.02	2.06
2	Fresh	0.543	0.050	94.1	6.3	0.01	0.89
	Frozen	0.544	0.073	92.6	6.3	0.03	3.33
3	Fresh	0.765	0.070	93.6	8.9	0.01	0.93
	Frozen	0.770	0.070	92.6	9.0	0.02	2.49
4	Fresh	0.506	0.050	93.7	5.9	0.01	1.33
	Frozen	0.530	0.052	92.7	6.2	0.04	3.82
5	Fresh	0.572	0.054	93.6	6.7	0.02	1.69
	Frozen	0.534	0.053	92.7	6.2	0.03	3.45
6	Fresh	0.172	0.033	91.0	2.0	0.04	3.75
	Frozen	0.176	0.033	89.0	2.0	0.09	9.68

Abbreviations: OD, optical density; NNC, Negative Neutralizing Control; NR, Neutralizing Reagent; NHS, Normal Human Serum; CO, Cutoff(0.086), Signal/CO, Ratio of Mean OD of NNC+ specimen to cutoff; SD, standard deviation; %CV, percent coefficient of variation.

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

**D. Sensitivity and Specificity for HIV-1 Subtype O**

The ability of HIV-1 p24 Antigen ELISA Test System 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 HIV-1 p24 Antigen Neutralization Kit.

**REFERENCES**

- 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*; 138; 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, Sarngadharan 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.
- 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.
- 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.
- National Committee for Clinical Laboratory Standards. Clinical Laboratory hazardous waste; proposed guidelines. NCCLS Document GP5-P. Villanova, PA: NCCLS, 1986.
- U.S Environmental Protection Agency. EPA guide for infectious waste management. Washington, DC: U.S. Environmental Protection Agency, Publication No. EPA/530-SW-86-014, 1986.
- 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).
- National Committee for Clinical Laboratory Standards. Proposed guideline: Specifications for immunological testing for infectious disease. NCCLS Document I/LA, Villanova PA; NCCLS, 1991.
- National Committee for Clinical Laboratory Standards. Approved guideline. Internal quality control testing; principle and definitions. NCCLS Document C24-A. Villanova PA; NCCLS, 1991.

**TRADEMARKS**

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