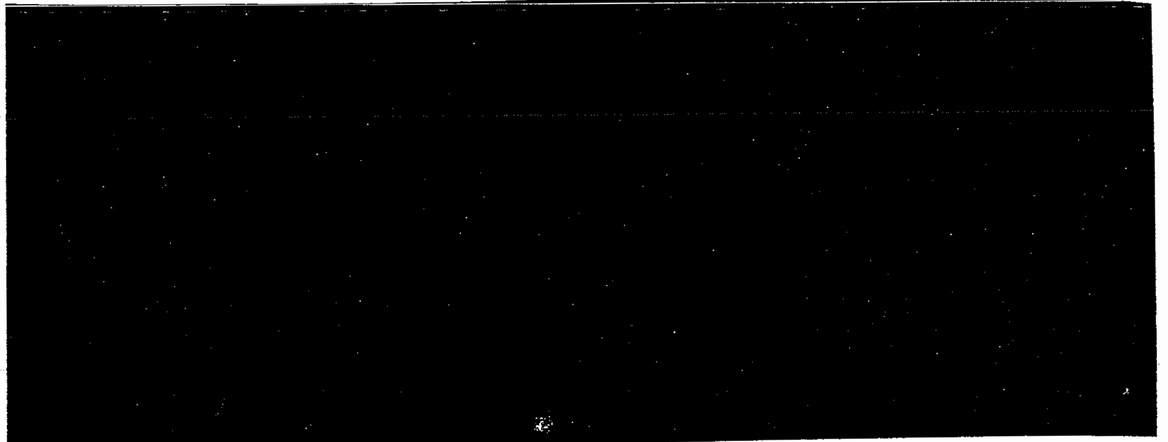
2401

ANTIBODY TO HUMAN IMMUNODEFICIENCY VIRUS TYPE 1

HIVAG-1

ABBOTT LABORATORIES
Diagnostics Division



83-4895/R3

© Abbott Laboratories, 1989

CUSTOMER SUPPORT CENTER (USA)
1-800/323-9100

HIVAG-1 ASSAY PROCEDURE for Serum and Plasma (See Preliminary Comments and Procedural Notes)

ABBOTT LABORATORIES
Diagnostics Division
Abbott Park, IL 60064

FIRST INCUBATION

1. Dispense 20 μL of Specimen Diluent to each well of reaction tray.
2. Dispense 200 μL of Specimen or Controls (3 Negative and 2 Positive) into bottom of appropriate wells of reaction tray.
3. Carefully add one bead to each well containing a specimen or control.
4. Apply cover seal. Gently tap the tray to remove trapped air bubbles.
5. Incubate at room temperature (15 to 30°C) for 16 to 20 hours.
6. Remove and discard cover seal. Aspirate the liquid and wash each bead with distilled or deionized water for a total rinse volume of 12 to 18 mL.
7. Remove all excess liquid from top of tray by aspiration or blotting.

SECOND INCUBATION

8. Dispense 200 μL of Antibody to HIV-1 (Rabbit) into each well containing a bead.
9. Repeat Step 4.
10. Incubate at 40 ± 2°C for 4 hours ± 10 minutes.
11. Repeat Step 6.
12. Repeat Step 7.

THIRD INCUBATION

13. Dispense 200 μL of Anti-Rabbit IgG Conjugate (Goat) into each well containing a Bead.
14. Repeat Step 4.
15. Incubate at 40 ± 2°C for 2 hours ± 10 minutes.
16. Repeat Step 6.
17. Repeat Step 7.

COLOR DEVELOPMENT

18. Immediately transfer the beads to properly identified assay tubes.
19. Pipette 300 μL of freshly prepared OPD Substrate Solution into two empty tubes (substrate blanks) and then into each tube containing a bead.
    NOTE: Prime Dispenser immediately prior to dispensing OPD Substrate Solution.
20. Cover and incubate at room temperature for 30 ± 2 minutes.
21. Add 1 mL of 1 *N* Sulfuric Acid to each tube.

READING

22. Blank Quantum Analyzer or spectrophotometer with a substrate blank at 492 nm.
23. Determine absorbance of controls and specimens at 492 nm.

List 7042 May, 1989 Printed in U.S.A.

NAME AND INTENDED USE

ƎHIVAG-1 is an *In Vitro* Enzyme Immunoassay for the Detection of Human Immunodeficiency Virus Type 1 (HIV-1) Antigen(s) in Human Serum or Plasma. It is intended to be used as an aid in the diagnosis and prognosis of patients with HIV-1 infection. **This test is not intended as a screen for donated blood or plasma.**

SUMMARY AND EXPLANATION OF THE TEST

Epidemiologic data indicate that the acquired immunodeficiency syndrome (AIDS) is caused by a virus transmitted by sexual contact, exposure to blood (including sharing contaminated needles and syringes) or certain blood products, or transmitted from an infected mother to her fetus or child during the perinatal period.[1-4] Human Immunodeficiency Virus Type 1 (also referred to as HIV-1) has been isolated from patients with AIDS and AIDS-related complex (ARC), and from asymptomatic persons at high risk for AIDS.[5] Detection of viable virus in blood and certain other body fluids can be achieved through complex procedures, such as co-culture of peripheral blood lymphocytes.[6] Direct evidence of the presence of the virus can also be achieved by testing for the presence of viral antigen(s) in serum and plasma.

Studies have shown that HIV-1 antigen is detectable in some infected individuals prior to HIV-1 antibodies. This observation has been made in all high risk groups studied.[7-14] A similar observation has been made in a very limited number of donors of source plasma who were bled at frequent intervals, prior to HIV-1 seroconversion.[15] Studies of donors of whole blood have failed to detect cases positive for HIV-1 antigen prior to antibody, presumably because of the low incidence of new HIV-1 infections in donors and the long interval between donations. Detection of HIV-1 antigen(s) has also been shown to correlate with the development of clinical complications related to HIV-1 infection.[16-19] Other uses for HIV-1 antigen testing which have been reported are monitoring the presence of HIV-1 antigen in co-cultures of peripheral blood lymphocytes[20,21] and monitoring treatment of HIV-1 infected individuals with antiviral drugs in research studies.[22-27]

ƎHIVAG-1 is an enzyme immunoassay for the detection of HIV-1 antigen(s) in human serum and plasma. Specimens with absorbance values less than the Cutoff Value are negative by ƎHIVAG-1. Specimens with absorbance values equal to or greater than the Cutoff Value are considered reactive, but should be retested in duplicate to determine whether the reactivity is a reproducible finding. Repeatably reactive specimens should be tested using ƎHIVAG-1 BLOCKING ANTIBODY. This procedure uses a specific antibody neutralization step followed by testing with ƎHIVAG-1 to indicate the presence of HIV-1 antigen(s). Specimens which are neutralized in this procedure are considered positive for HIV-1 antigen(s). Conversely, specimens which are not neutralized in this procedure are considered negative.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

ƎHIVAG-1 is a "sandwich" solid phase enzyme immunoassay used to detect HIV-1 antigen(s).

- HIV-1 virions, when present in the test sample, are disrupted by the addition of sample diluent containing Triton X-100.
- Polystyrene beads coated with human antibody (Ab) to HIV-1 are incubated with either a specimen or Control. HIV-1 antigen(s) (Ag) in the specimen binds to the bead. After incubation, unbound materials are aspirated and the beads are washed.
- Rabbit antibody to HIV-1 is then incubated with the bead and binds to the HIV-1 Ag. Unbound materials are aspirated and the beads are washed.
- Goat antibody to rabbit IgG conjugated with horseradish peroxidase (anti-Rabbit IgG: HRPO) is incubated with the bead and binds to the rabbit antibody. Unbound materials are aspirated and the beads are washed.
- Next, o-Phenylenediamine (OPD) Solution containing hydrogen peroxide is added to the bead and, after incubation, a yellow-orange color develops in proportion to the amount of HIV-1 antigen bound to the bead.

ƎHIVAG-1 BLOCKING ANTIBODY utilizes the principle of specific antibody neutralization to confirm the presence of HIV-1 antigen.

- The Blocking Antibody (human antibody to HIV-1) is pre-incubated with a **repeatably reactive** specimen or controls. If HIV-1 antigen is present in the specimen, it will form an immune complex with the blocking antibody, thereby reducing the antigenic sites available for subsequent reaction.
- The sample and controls are then tested with ƎHIVAG-1. The neutralized (blocked) HIV-1 antigen in the specimen is prevented from binding to the HIV-1 antibody coated bead in the first incubation. This results in a reduction of signal when compared to a non-neutralized specimen in which Control Reagent is used in place of the blocking antibody. By definition, a specimen is considered positive if the reduction in absorbance of the neutralized specimen is 50% or greater when compared to the non-neutralized control.

REAGENTS

No. 7042, ƎHIVAG-1 KIT, (100 /1000 Tests)

(1) 100/1000 Antibody to HIV-1 (Human) Coated Beads. (Inactivated).
(2) 1 Vial (20 mL)/2 Bottles (100 mL each) Anti-Rabbit IgG Conjugate. Antibody to Rabbit IgG (Goat): Peroxidase (Horseradish). Minimum Concentration: 0.01 μg/mL. Preservatives: Gentamycin/Thimerosal.
(3) 1 Vial (6.5 mL)/2 Vials (6.5 mL each) Positive Control HIV-1 Antigen(s). Inactivated. Preservative: 0.1% Sodium Azide.
(4) 1 Vial (9.5 mL)/2 Vials (9.5 mL each) Negative Control. Recalcified human plasma nonreactive for HIV-1 Antigen(s), HBsAg, and Antibody to HIV-1. Preservative: 0.1% Sodium Azide.
(5) 1 Vial (20 mL)/2 Bottles (100 mL each) Antibody to HIV-1 (Rabbit). Minimum Concentration: 0.1 μg/mL. Preservative: 0.1% Sodium Azide.
(6) 1 Bottle (30 mL)/2 Bottles (30 mL each) Specimen Diluent containing Triton X-100. Preservative: 0.1% Sodium Azide.
(9) 1 Bottle (10 tablets)/2 Bottles (40 tablets each) OPD (o-Phenylenediamine • 2 HCl) tablets. OPD/Tablet: 12.8 mg.
(10) 1 Bottle (55 mL)/2 Bottles (220 mL each) Diluent for OPD (o-Phenylenediamine • 2 HCl). Citrate-Phosphate Buffer containing 0.02% Hydrogen Peroxide.

The Stopping Reagent is provided as an accessory to the ƎHIVAG-1 Kit and consists of:

(11) 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:

1. Pipette 300 μL of OPD Substrate Solution into 5 EIA reaction tubes or acid washed/distilled or deionized water rinsed tubes.
2. Add 2 mL of the 1 *N* Sulfuric Acid under test to each of the 5 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 0.08 at "0 TIME" and
   b. a difference of less than 0.03 units in the values obtained at "0 TIME" and "120 MIN".

ƎHIVAG-1 Blocking Antibody (No. 7043) is available as an accessory to the ƎHIVAG-1 kit:

No. 7043, ƎHIVAG-1 BLOCKING ANTIBODY, 25 TESTS

(1) 1 Vial (3 mL) Antibody to HIV-1 (Human). Inactivated (Solution A). Minimum titer: 1:2. Preservative: 0.1% Sodium Azide.
(2) 1 Vial (5 mL) Control Reagent. (Solution B). Human plasma negative for HIV-1 Antigen(s), HBsAg and Antibody to HIV-1. Preservative: 0.1% Sodium Azide.

WARNINGS AND PRECAUTIONS

FOR IN VITRO DIAGNOSTIC USE

Safety Precautions

CAUTION: Handle all ƎHIVAG-1 and ƎHIVAG-1 BLOCKING ANTIBODY biological materials as though capable of transmitting infection.[28] No known test method can offer complete assurance that products derived from human blood will not transmit infection. Therefore all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices. The Positive Control has been inactivated by treatment with non-ionic detergent. THE BLOCKING ANTIBODY HAS BEEN INACTIVATED BY HEAT TREATMENT.

1. Do not pipette by mouth.
2. Do not smoke, eat, or drink in areas in which specimens or kit reagents are handled.
3. Wear disposable gloves while handling kit reagents or specimens and thoroughly wash hands afterward.
4. Avoid splashing or forming an aerosol.
5. Avoid contact of OPD and sulfuric acid with skin and mucous membranes. If these reagents come into contact with skin, wash thoroughly with water.
6. Dispose of all specimens and materials used to perform the test as if they contained infectious agents. The preferred method of disposal is autoclaving for a minimum of one hour at 121.5°C. Disposable materials may be incinerated. Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite.[29] Allow 30 minutes for decontamination to be completed.
   CAUTION: Liquid waste containing acid must be neutralized with a proportional amount of base prior to the addition of sodium hypochlorite.[30]
7. SPILLS: Spills should be wiped up thoroughly using either an iodophor disinfectant or sodium hypochlorite solution.

Iodophor Disinfectant:

- Iodophor Disinfectant should be used at a dilution providing at least 100 ppm available iodine. Hy Sine, Ioprep, Mikroklene and Wescodyne are proprietary iodophors; the latter may be used at approximately a 1:100 to 1:150 final dilution in water. Neutralization of acid-containing spills is not required.[31-33]

Sodium Hypochlorite:

- Non acid-containing spills should be wiped up thoroughly with a 5% sodium hypochlorite solution.
- Acid-containing spills should be wiped dry. Spill areas should then be wiped with a 5% sodium hypochlorite solution.

Materials used to wipe up spills should be added to biohazardous waste matter for proper disposal.

8. 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, flush drains thoroughly 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 recommends the following: (1) siphon liquid from 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 ƎHIVAG-1 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 into 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.

Five to ten minutes prior to Color Development, prepare the OPD Substrate Solution by dissolving the OPD (o-Phenylenediamine • 2 HCl) Tablet 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 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 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 and **must not be exposed to strong light.**
3. Just prior to dispensing for Color Development, swirl the container gently to obtain a homogeneous solution, and remove air bubbles from tubing.

→ over

3. Calculation of the Cutoff Value.
   Calculate the Cutoff Value using the following equation:

   Cutoff Value: serum or plasma samples = $NC\bar{x}$ + 0.050

   Example: $NC\bar{x}$ = 0.051
   + 0.050

   Cutoff Value = 0.101

4. Determination of the P–N Value.
   Calculate the P–N Value by subtracting the $NC\bar{x}$ from the $PC\bar{x}$.

   Example: $PC\bar{x}$ = 1.463
   $NC\bar{x}$ = 0.051

   P–N = 1.412

   For the run to be valid, the P–N must be greater than or equal to 0.400. If not, technique or deterioration of reagents may be suspect and the run should be repeated.

INTERPRETATION OF RESULTS FOR SERUM OR PLASMA

1. Specimens with absorbance values less than the Cutoff Value are considerad negative by the criteria of ƎHIVAG-1.
2. Specimens with absorbance values greater than or equal to the Cutoff Value are considered reactive and should be repeated in duplicate using the original sample source. If both replicates are less than the cutoff Value, the sample should be classified as negative. If either of the replicates are greater than or equal to the Cutoff Value, the specimen should be classified as repeatably reactive for HIV-1 antigen.
3. Repeatably reactive specimens should be further tested with ƎHIVAG-1 BLOCKING ANTIBODY (refer to ƎHIVAG-1 BLOCKING ANTIBODY TEST procedure). Repeatably reactive specimens which are neutralized in this procedure are considered positive for HIV-1 antigen. Repeatably reactive specimens which do not neutralize are considered negative for HIV-1 antigen.

PROCEDURE FOR TESTING CELL CULTURE SUPERNATANTS

Detection of HIV-1 antigen(s) has been useful in monitoring various types of HIV-1 cultures (infectious plasma, peripheral blood lymphocyte co-culture or infected cell lines).[6,21]

Materials Required But Not Provided

- Cell Culture Control for testing uninfected cell culture samples. This should be the same cell culture material (e.g. tissue culture medium such as RPMI-1640 containing fetal calf serum) used for infected cell culture samples.

Preliminary Comments

1. The following procedure may be used to test cell culture samples.
2. When testing or neutralizing cell culture samples, additional 3 Cell Culture Controls from uninfected cell culture samples must be assayed.

PROCEDURE

1. DISPENSE 20 µL OF SPECIMEN DILUENT TO EACH WELL OF THE REACTION TRAY.
2. DISPENSE 20 µL OF THE NEGATIVE CONTROL INTO EACH WELL THAT WILL CONTAIN POSITIVE CONTROL (2), NEGATIVE CONTROL (3), CELL CULTURE CONTROL (3) OR CELL CULTURE SAMPLE.
3. DISPENSE 200 µL OF EACH SPECIMEN OR CONTROL INTO BOTTOM OF APPROPRIATE WELLS OF THE REACTION TRAY.
4. PROCEED TO STEP 3–23 OF THE ƎHIVAG-1 PROCEDURE FOR SERUM OR PLASMA.

CALCULATION OF RESULTS FOR CELL CULTURE SUPERNATANTS

1. Calculation of the Cell Culture Control Mean ($CCC\bar{x}$)
   Example:

   Cell Culture Control

Sample No.	Absorbance
1	0.091
2	0.098
3	0.087
Total	0.276

$$\frac{\text{Total Absorbance}}{3} = \frac{0.276}{3} = 0.092\ (CCC\bar{x})$$

2. Calculation of the Cutoff Value
   Calculate the Cutoff Value using the following equation

   Cutoff Value: $CCC\bar{x}$ + 0.050

   Example:

   $CCC\bar{x}$ = 0.092
   + 0.050

   0.142

INTERPRETATION OF RESULTS FOR CELL CULTURE SUPERNATANTS

1. Specimens with absorbance values less than the Cutoff Value are considered negative by the criteria of ƎHIVAG-1.
2. Specimens with absorbance values greater than or equal to the Cutoff Value are considered reactive and should be repeated in duplicate using the original sample source. If both replicates are less than the Cutoff Value, the original result should be classified as negative. If either of the replicates are greater than or equal to the Cutoff Value, the specimen should be classified as repeatably reactive for HIV-1 antigen.
3. All repeatably reactive specimens should be further tested with ƎHIVAG-1 BLOCKING ANTIBODY (refer to HIVAG-1 BLOCKING ANTIBODY TEST procedure). Repeatably reactive specimens which are neutralized in this procedure are considered positive for HIV-1 antigen. Repeatably reactive specimens which do not neutralize are considered negative for HIV-1 antigen. As an alternative to the blocking test, an independent sample taken subsequently from the same culture may be run. If this second sample is repeatably reactive, the cell culture supernatant may be classified as positive.

ƎHIVAG-1 BLOCKING ANTIBODY TEST PROCEDURE

Preliminary Comments

1. This procedure may be used to test repeatably reactive specimens of serum, plasma or cell culture samples.
2. Three Negative and four Positive Controls (two to be neutralized, two as non-neutralized controls) must be assayed with each run of unknowns. For each unknown specimen to be assayed, four samples are run (two to be neutralized, two as non-neutralized controls). Ensure that all reaction trays are subjected to the same process and incubation times and handling.
   NOTE: WHEN TESTING CELL CULTURE SAMPLES AN ADDITIONAL 3 CELL CULTURE CONTROLS FROM UNINFECTED CELL CULTURE SAMPLES MUST BE ASSAYED.

3. If the specimen to be tested has an absorbance value greater than or equal to 2.000 in ƎHIVAG-1, the specimen must be diluted 1:1 with Negative Control prior to testing by the Blocking Procedure. However, if a 1:1 dilution of the specimen is tested and the absorbance value of the non-neutralized specimen control is less than $NC\bar{x}$ + 0.040, the Blocking Procedure must be repeated using the specimen undiluted.

PROCEDURE

1. DISPENSE 20 µL OF SPECIMEN DILUENT TO EACH WELL OF REACTION TRAY.
2. PIPETTE 200µL OF EACH CONTROL INTO BOTTOM OF APPRDPRIATE WELLS OF REACTION TRAY (3 NEGATIVE CONTROLS AND 4 POSITIVE CONTROLS).
   NOTE: IF CELL CULTURE SPECIMENS ARE BEING TESTED RUN 3 NEGATIVE CONTROLS, 4 POSITIVE CONTROLS AND 3 CELL CULTURE CONTROLS.
3. PIPETTE 200µL OF EACH SPECIMEN BEING TESTED INTO THE BOTTOM OF 4 WELLS.
4. DISPENSE 50 µL OF BLOCKING ANTIBODY (SOLUTION A), TO 2 OF THE 4 WELLS CONTAINING POSITIVE CONTROL. TO THE REMAINING 2 POSITIVE CONTROL WELLS AND ALL 3 NEGATIVE CONTROL (OR CELL CULTURE CONTROL) WELLS, DISPENSE 50 µL CONTROL REAGENT (SOLUTION B).
5. TO 2 OF THE 4 SPECIMEN WELLS, DISPENSE 50 µL BLOCKING ANTIBODY (SOLUTION A). TO THE REMAINING 2 WELLS, DISPENSE 50 µL OF CONTROL REAGENT (SOLUTION B).
6. APPLY COVER SEAL. TAP TRAY GENTLY TO FACILITATE MIXING.
7. INCUBATE AT ROOM TEMPERATURE FOR 2 HOURS ± 10 MINUTES.
8. PROCEED TO STEPS 3–23 OF THE ƎHIVAG-1 PROCEDURE FOR SERUM OR PLASMA.

READING RESULTS FOR ƎHIVAG-1 BLOCKING ANTIBODY TEST

Laboratories using Quantum™ II or Quantumatic™ should determine absorbance as outlined below:

QUANTUM II*	QUANTUMATIC*
Edit Module A containing the AUSZYME CONFIRMATORY PROTOCOL (MODE 2.1)	For 7523-29 and below, edit the AUSZYME CONFIRMATORY Protocol as follows:
For all Modules, EDIT the following line:	NAME: HIV-1 AG NEUTRALIZATION NO OF NEG CONTROLS = 3 MIN NEG CONTROL = –0.010
Line 15: Change from — 0.025 to 0.040.	MAX NEG CONTROL = 0.150 NO < POS CTL + CTL SERUM> = 2 NO < POS CTL + ANTIBODY> = 2
OR	MIN CONTROL DIFF = 0.400 MAX CONTROL DIFF =
USE MODE 0	MIN % NEUTRALIZATION = 50.0% NO < PAT + CTL SERUM > Rep = 2 NO < PAT + ANTIBODY > Rep = 2 MIN PAT ABSORB DIFF = NEUT. CUTOFF % = 50.0% CUTOFF CONSTANT = 0.040

*NOTE: For Automatic Data Reduction on the Quantum II or Quantumatic, the non-neutralized (Solution B) duplicates (for Positive Control and unknowns) must be read immediately before the respective neutralized (Solution A) duplicates. The Quantum II MAX NEG CTL specification is set at 0.100 and is different from that used on Quantumatic.

Laboratories using an instrument other than a Quantum Analyzer should read this assay as follows:

- Read the absorbances for each Control and specimen.
- Rinse cuvettes thoroughly with distilled or deionized water between the reading of each specimen.
- Refer to RESULTS section for calculations.

TM—Trademark

CALCULATION OF RESULTS FOR ƎHIVAG-1 BLOCKING ANTIBODY TEST

1. Calculate the mean absorbance value (A_{492}) for the Negative Control or Cell Culture Control as described under calculation of results for ƎHIVAG-1.
2. Calculation of the Positive Control Mean ($PC\bar{x}$).
   Determine the mean of both the two neutralized and two non-neutralized Positive Control Values.

Example:

Positive Control (Non-Neutralized, Solution B)

Sample No.	Absorbance
1	1.485
2	1.493
	2.978

$$\frac{\text{Total Absorbance}}{2} = \frac{2.978}{2} = 1.489\ (PC\bar{x}\ \text{Solution B})$$

Positive Control (Neutralized, Solution A)

Sample No.	Absorbance
1	0.041
2	0.045
	0.086

$$\frac{\text{Total Absorbance}}{2} = \frac{0.086}{2} = 0.043\ (PC\bar{x}\ \text{Solution A})$$

3. Calculation of the Specimen Mean ($S\bar{x}$)
   Determine the means of both the two neutralized and two non-neutralized samples.

Example:

Sample (Non-Neutralized)

Sample No.	Absorbance
1	0.553
2	0.545
	1.098

$$\frac{\text{Total Absorbance}}{2} = \frac{1.098}{2} = 0.549\ (S\bar{x}\ \text{Solution B})$$

Sample (Neutralized)

Sample No.	Absorbance
1	0.044
2	0.038
	0.082

$$\frac{\text{Total Absorbance}}{2} = \frac{0.082}{2} = 0.041 \text{ (S}\bar{x}\text{ Solution A)}$$

4. Calculation of the Cutoff Value.
   Calculate the Cutoff Value using the following equation:
   Cutoff Value: Serum or Plasma samples = $NC\bar{x}$ + 0.040*
   Cell Culture samples = $CCC\bar{x}$ + 0.040

Example:

	$NC\bar{x}$ = 0.051	$CCC\bar{x}$ = 0.092
	+ 0.040	+ 0.040
Cutoff Value =	0.091	0.132

*See Interpretation of Results for HIVAG-1 Blocking Antibody Test.

5. Calculation of Percent Reduction in Absorbance Values.
   Determine the percent reduction of the neutralized Positive Control and for each neutralized specimen using the following equation:

$$\% \text{ Reduction} = \frac{A_{492}\text{ Solution B} - A_{492}\text{ Solution A}}{A_{492}\text{ Solution B} - A_{492}\text{ (NC}\bar{x}\text{ or CCC}\bar{x}\text{)}} \times 100\%$$

Example: **Mean Absorbance**

Negative Control	0.051
Positive Control + Solution B	1.489
Positive Control + Solution A	0.043
Specimen + Solution B	0.549
Specimen + Solution A	0.041

$$\text{Positive Control: } \frac{1.489 - 0.043}{1.489 - 0.051} \times 100 = \mathbf{100.6\%} \text{ Neutralization}$$

$$\text{Specimen: } \frac{0.549 - 0.041}{0.549 - 0.051} \times 100 = \mathbf{102.0\%} \text{ Neutralization}$$

INTERPRETATION OF RESULTS FOR HIVAG-1 BLOCKING ANTIBODY TEST

The test may be considered valid if the net absorbance of the Positive Control (A_{492} $PC\bar{x}$ Solution B − A_{492} $NC\bar{x}$ or $CCC\bar{x}$) is greater than or equal to 0.400 and the Positive Control is neutralized by 50% or greater.

A specimen is considered positive if BOTH of the following criteria are met:

1. The mean absorbance value of the specimens (or diluted specimens) plus Solution B is equal to or greater than the Negative Control Mean or Cell Culture Control Mean plus 0.040.*
2. The percent reactivity of the specimen (or diluted specimens) is reduced by 50% or greater.

*The cut-off constant used in the blocking antibody test is different from the constant used in the HIVAG-1 procedure for serum or plasma. This compensates for the dilution factor introduced by the addition of the blocking reagent (Solution A) or control reagent (Solution B).

LIMITATIONS OF THE PROCEDURE

The HIVAG-1 and HIVAG-1 BLOCKING ANTIBODY procedures and Interpretation of Results must be closely followed. Because the procedures were designed to test individual serum, plasma and cell culture supernatants, insufficient data are available to interpret tests performed on other body fluid specimens, pooled blood or processed plasma, or products made from such pools. Testing of these specimens is not recommended.

HIV-1 ANTIGEN IS A LOW SENSITIVITY MARKER FOR DETECTION OF HIV INFECTION SINCE IT IS PRESENT ONLY TRANSIENTLY PRIOR TO SEROCONVERSION AND LATER IT IS COMPLEXED BY SPECIFIC ANTIBODIES. ANTIGEN TESTING SHOULD NOT BE USED IN LIEU OF ANTIBODY TESTING AS A SCREEN FOR HIV INFECTION. In comparison with antibody testing, antigen testing will only detect approximately 50% of AIDS, 30% of ARC and 10% of asymptomatic HIV infections. (See SPECIFIC PERFORMANCE CHARACTERISTICS, Section III, Sensitivity).

The HIVAG-1 procedure for detection of HIV-1 antigen(s) has been demonstrated to have a very high specificity of 99.82%. (See SPECIFIC PERFORMANCE CHARACTERISTICS, Section II, Specificity). Nevertheless, the predictive value of a positive test is strongly influenced by the prevalence of the condition in the population tested.

IN LOW RISK POPULATIONS, where the rate of HIV-1 infection may not exceed 0.1%, the rate of antigen positivity could be as low as 0.01%. Assuming a test sensitivity of 100%, the positive predictive value of a repeatedly reactive test would be only 5.9%, i.e. only six tests per 100 would be true positives. For this reason, IT IS STRONGLY RECOMMENDED THAT THE HIVAG-1 BLOCKING ANTIBODY PROCEDURE BE PERFORMED ON ALL CLINICAL SAMPLES THAT ARE REPEATABLY REACTIVE.

IN POPULATIONS WITH KNOWN HIV-1 INFECTION, the predictive value of a repeatably reactive test is significantly greater. For example, in asymptomatic seropositive persons, HIV-1 antigen(s) may be present in approximately 10% of cases. Again, assuming 100% test sensitivity, the predictive value of a repeatably reactive test would be 98.6%, i.e. fewer than two false positives per 100 repeatable reactives.

The sensitivity and specificity of the HIVAG-1 BLOCKING ANTIBODY procedure are not known, however, estimates can be obtained from the clinical data by applying the binomial distribution. In clinical studies performed in low risk populations (Table I), the neutralization test was negative for 137/137 repeatably reactive (presumed false reactive) samples giving a 95% confidence range for specificity of 97.8% to 100%. In clinical studies performed in known infected individuals (Table III, Study 1), the HIVAG-1 BLOCKING ANTIBODY test was positive in 67/67 repeatably reactive (presumed true positive) samples giving a 95% confidence range for sensitivity of 95.9% to 100%.

AIDS and AIDS-related conditions are clinical syndromes and their diagnosis can only be established clinically[34] HIVAG-1 and HIVAG-1 BLOCKING ANTIBODY results should be used as an adjunct to other information available to the physician[35] HIVAG-1 testing alone cannot be used to diagnose AIDS, even if the recommended investigation of reactive specimens indicates that HIV-1 antigen is present. A negative test result at any point in the investigation of individual subjects does not preclude the possibility of exposure to or infection with HIV-1.

SPECIFIC PERFORMANCE CHARACTERISTICS

I. Precision

Assay reproducibility for serum and plasma was determined by testing 5 member HIV-1 antigen dilution panels. Each panel member was tested in replicates of 5 over 3 consecutive runs. The percent coefficient of variation (%CV) was calculated for each panel member and assay controls. In these studies, the %CV ranged from 3.3% to 10.6% within run (intra-assay) and from 5.8% to 21.7% between runs (inter-assay).

II. Specificity

The percentage of specimens found positive with HIVAG-1 was determined by testing serum and plasma specimens collected from healthy control subjects. Data presented in Table I are from 75,139 specimens tested at 3 sites. Specimens were screened and determined to be negative for antibody to HIV-1.

137 (0.18%) were found to be repeatably reactive with HIVAG-1. The specificity of HIVAG-1 is estimated to be 99.82% prior to neutralization.

Table I

Detection of HIV-1 Antigen(s) in Serum and Plasma from Healthy Subjects.

Site	Type of Donor	Type of Sample	Number Tested	Initial Reactive	%	Repeat Reactive	%	Neutralized
A	Whole blood	serum	5,187	15	0.29	5	0.10	0
		serum	31,751	97	0.31	23	0.07	0
B	Apheresis	plasma	3,998	16	0.40	11	0.28	0
C	Whole blood	serum	34,203	223	0.65	98	0.29	0
TOTAL			75,139	351	0.47	137	0.18	0

III. Sensitivity

In the absence of alternative assays for HIV-1 antigen detection, sensitivity cannot be formally assessed.

Sensitivity of the test as a diagnostic tool for HIV-1 infection is limited by the fact that HIV-1 antigen(s) can only be detected for a brief time before antibodies develop[15] or when HIV-1 antigen is in excess of antibodies. Estimates of the sensitivity of the test for diagnosis of infection were obtained from studies performed using HIVAG-1 to determine the prevalence of HIV-1 antigen(s) in the serum or plasma of patients clinically diagnosed as having AIDS, AIDS-related complex (ARC) or from asymptomatic seropositive individuals (Table II). These data indicate that the prevalence of HIV-1 antigen in serum and plasma correlates with the clinical severity of infection. The cumulative prevalences are 45% in AIDS; 23% in ARC and 13% in asymptomatic antibody positive individuals. Compared with antibody testing, HIVAG-1 has a very low sensitivity for detection of HIV-1 infection and should not be used in lieu of the antibody test.

The sensitivity of the test for detecting HIV-1 antigen(s) which is not complexed by antibodies was assessed by the use of dilution panels consisting of antigen from HIV-1 grown in cell culture. The sensitivity of HIVAG-1 for purified viral p24 (>95% purity) is 5–20 pg/ml. The sensitivity for detection of a viral lysate is 50 ± 20 pg/ml. Viral lysate consists of a mixture of HIV-1 antigens and other proteins. The sensitivity is dependent on the relative proportions of viral antigens which may vary from lot to lot. Values indicated above are based on reference material produced at Abbott Laboratories.

Table II

Prevalence of HIV-1 Antigen(s) in Clinical Groups[a]

Group	No. of Specimens	Initially Reactive	Repeatably Reactive	% Repeatably Reactive	Neutralized
AIDS	40	18	18	45.0	18
AIDS Related Complex (ARC)	30	7	7	23.3	7
Asymptomatic[b]	30	4	4	13.3	4
High Risk for[c] HIV (IVDA)	25	0	0	0.0	0
Other Diseases[d]	43	0	0	0.0	0

[a] Data collected in 1989 on serum or plasma samples tested with Triton X-100.
[b] All specimens were seropositive for antibody to HIV-1.
[c] All specimens were seronegative for antibody to HIV-1.
[d] Other diseases include: 2 systemic lupus erythematosus, 7 gram-positive infections, 10 E. coli infections, 10 ANA (anti-nuclear antibody), 9 autoimmune disorders, 3 rheumatoid factor, 2 rheumatoid factor + ANA.

IV. HIV-1 Antigen as a Prognostic Marker

Homosexual Males

Data from 3 prospective studies of 498 HIV-1 antibody positive homosexual males are shown in Table III which indicate the use of HIV-1 antigen as a marker for predicting clinical complications of HIV-1 infection. All subjects were classified as either asymptomatic (CDC II) or with generalized lymphadenopathy (CDC III) on entry. After stratification according to HIV-1 antigen status, actuarial progression and odds ratio analysis[36] were carried out to evaluate the significance of the association of HIV-1 antigen with the occurrence of AIDS over 2 to 3 years. The risk of developing AIDS over this period is significantly higher in HIV-1 antigen positive individuals (39–75%) than in HIV-1 antigen negative individuals (8–29%). The Odds Ratio to AIDS for HIV-1 antigen positive individuals relative to HIV-1 antigen negative subjects ranged from 5.6–6.4. However, HIV-1 antigen negative individuals were still at risk of developing AIDS during the same period.

Hemophiliacs

A cohort of 66 hemophiliacs was followed prospectively since the time of seroconversion. Over an average of 60 months of follow-up, 24 patients (36%) developed HIV-1 antigen. Table IV summarizes the occurrence of HIV related clinical symptoms (candidiasis, Herpes zoster, Herpes simplex or severe bacterial infection) in 12 of the 24 hemophiliacs which were HIV-1 antigen positive and 5 of the 42 hemophiliacs which were HIV-1 antigen negative. The odds ratio of developing clinical symptoms for HIV-1 antigen positive hemophiliacs versus antigen negative hemophiliacs is 5.4. The average time interval between seroconversion and occurrence of HIV-1 related symptoms is 31 ± 18 months for HIV-1 antigen positive hemophiliacs and 50 ± 7 months for HIV-1 antigen negative hemophiliacs (p<0.001 by Wilcoxon rank test). Among the 12 HIV-1 antigen positive hemophiliacs who developed symptoms, 8 became symptomatic 24 ± 17 months after the occurrence of HIV-1 antigen and 4 became symptomatic 0–10 months prior to antigenemia.

17

TABLE III

Actuarial Progression Rates and Odds Ratios to AIDS Relative to HIV-1 Antigen Status[a]

18

Population	Study	Number of Subjects	Percent HIV-1 Antigen Positive	Time of Follow-up (months)[b]	Actuarial Risk (%) of developing AIDS or symptoms over Study Period: Antigen Positive	Actuarial Risk (%) of developing AIDS or symptoms over Study Period: Antigen Negative	Odds Ratio	95% Confidence Interval	p Value
Homosexual males[c]	1	70	34	24	75	29	5.6	1.9–15.9	<.001
	2[d]	226	15	36	67	16	6.4	3.1–13.1	<.0001
	3[e]	202	19	33	39	8	6.2	2.6–14.9	<.0001

[a] These data were generated using a test system similar to that described but in the absence of Triton X-100 in the diluent. In-house studies (data not shown) indicate that the use of Triton X-100 would not change these results.

[b] This value represents a maximum.

[c] Criteria for stratification as antigen positive:

Study 1 – One neutralizable HIV-1 antigen positive specimen prior to the development of AIDS.

Study 2 & 3 – Repeatably reactive HIV-1 antigen specimen on entry; at which time all individuals were asymptomatic.

[d] Data from study 2 were published in reference 17.

[e] Data from study 3 were published in reference 18.

TABLE IV

Development of HIV-1 related clinical symptoms in 66 hemophiliacs followed since seroconversion[a]

Time of follow up (m)	0	6	12	18	24	30	36	42	48	54	60
HIV-1 antigen[b]		59[c]		51[c]	53		61[c]	49	50	57	
Positive patients (N = 12)		63[c]			65				52	64	
									54		
HIV-1 antigen[d]								20	27	39	33
Negative patients (N = 5)											12

[a] Numbers correspond to patient ID and represent individuals who developed symptoms during the preceding six month interval. Only patients who developed symptoms are presented.

[b] 12 hemophiliacs were HIV-1 antigen positive and did not develop symptoms over the 60 months of study.

[c] Hemophiliacs who developed symptoms 0–10 months prior to antigenemia.

[d] 37 HIV-1 antigen negative hemophiliacs did not develop symptoms.

19 BIBLIOGRAPHY

1. Chamberland, M.E., Castro, K.G., Haverkos, H.W., Miller, B.I., Thomas, P.A., Reiss, R., Walker, J., Spira, T.J., Jaffe, H.W., and Curran, J.W., Acquired Immunodeficiency Syndrome in the United States: An Analysis of Cases Outside High-Incidence Groups. Ann. Int. Med. 101: 617-623, 1984.
2. Curran, J.W., Lawrence, D.N., Jaffe H., Kaplan, J.E., Zyla L.D., Chamberland M., Weinstein, R., Lui, K.J., Schonberger, L.B., Spira, T.J., Alexander, W.J., Swinger, G., Ammann, A., Solomon, S., Auerbach, D., Mildvan, D., Stoneburner, R., Jason, J.M., Haverkos, H.W., and Evatt, B.L., Acquired Immunodeficiency Syndrome (AIDS) Associated with Transfusions. New Eng. J. Med. 310:69-75, 1984.
3. Scott, G.B., Buck, B.E., Laterman, J.G., Bloom, F.L., and Parks, W.P., Acquired Immunodeficiency Syndrome in Infants. New Eng. J. Med. 310:76-81, 1984.
4. Feorino, P.M., Jaffe, H.W., Palmer, E., Peterman, T.A., Francis, D.P., Kalyanaraman, V.S., Weinstein, R.A., Stoneburner, R.L., Alexander, W.J., Raevsky, C., Getchell, J.P., Warfield, D., Haverkos, H.W., Kilbourne, B.W., Nicholson, J.K.A., and Curran, J.W. Transfusion-Associated Acquired Immunodeficiency Syndrome. New Eng. J. Med. 312:1293-1296, 1985.
5. Gallo, R.C., Salahuddin, S.Z., Popovic, M., Shearer, G.M., Kaplan, M., Haynes, B.F., Palker, T.J., Redfield, R., Oleske, J., Safai, B., White, G., Foster, P., and Markham, P.D., Frequent Detection and Isolation of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and at Risk for AIDS. Science 224:500-502, 1984.
6. Salahuddin, Z.F., Markham, P.D., Popovic, M., et. al. Isolation of Infectious Human T Cell Leukemia/Lymphotropic Virus Type 3 (HTLV-III) from Patients with AIDS (AIDS) or AIDS related Complex (ARC) and from healthy carriers: a study of risk groups and tissue sources. Proc. Natl. Acad. Sci. 82: 5530-5534, 1985.
7. Goudsmit, J., De Wolf, F., Paul, D.A., Epstein, L.G., Lange, J.M.A., Krone, W.J.A., Speelman, H., Wolters, E.C., Van der Noordaa, J., Oleske, J.M., Van der Helm, H.J., Coutinho, R.A. Expression of human immunodeficiency virus antigen (HIV-Ag) in serum and cerebrospinal fluid during acute and chronic infection. Lancet, 2, 177-80, 1986.
8. Allain, J.P., Laurian, Y., Paul, D.A., Senn, D. Serological markers in early stages of human immunodeficiency virus Infection in hemophiliacs. Lancet, 2, 1233-38, 1986.
9. Joller-Jamelka, H., Grob, P. HIV-serologic in Fruhphase einer HIV-infection. Schweizerische Medizinische Wochenschrift, 117, 1283-88, 1987.
10. Kessler, H.H., Blaauw, B., Spear, J., Paul, D.A., Falk, L.A., Landay, A. Diagnosis of human immunodeficiency virus infection in seronegative homosexuals presenting with an acute viral syndrome. JAMA, 258, 1198-99, 1987.
11. Gaines, H., Albert, J., Von Sydow, M., Sonnerberg, A., Chiodi, F., Ehrust, A., Strannegard, O., Asjo, B. HIV antigenemia and virus isolation from plasma during primary HIV infection. Lancet, 1, 1317-18, 1987.
12. Bowen, A., Lobel, S.A., Carvana, R.J., Laffell, M.S., House, M.A., Riesing, J.P., Humphries, A.L. Transmission of human immunodeficiency virus (HIV) by transplantation. Clinical aspects and time course analysis of viral antigenemia and antibody production. Ann Intern Med, 108, 46-48, 1988.
13. Von Sydow, M., Gaines, H., Sonnerborg, A., Forsgran, M., Pehrson, P.O., Strannegard, O. Antigen detection in primary HIV infection. Brit Med J, 298, 238-40, 1988.
14. Simmonds, P., Lainson F.A.L., Cuthbert, R., Steel, C.M., Peutherer, J.F. HIV antigen and antibody detection: variable response to the Edinburgh haemophiliac cohort. Brit Med J, 298, 593-98, 1988.
15. Stremer, S.L., Heller, J.S., Coombs, R.W., et al. Detection of HIV Markers Prior to IgG Antibody Seropositivity in Plasma Donors. JAMA 282: 64-89, 1989.
16. Allain, J.P., Laurian, Y., Paul, D.A., Verroust, F., Leuther, M., Gazengel, C., Senn, D., Larrieu, M.J., and Bosser, C. Long-term Evaluation of HIV-1 Antigen and Antibodies to p24 and gp41 in Patients with Hemophilia. New Eng. J. Med., 317:1114-1121, 1987.
17. Moss, A.R., Bacchetti, P., Osmond, D., Krampf, W., Chaisson, R.E., Stites, D., Wilber, J., Allain, J.P., and Carlson, J. Seropositivity for HIV-1 and the Development of AIDS or AIDS Related Condition: Three Year Follow-up of the San Francisco General Hospital Cohort. Brit. Med. J. 296:745-750, 1988.
18. DeWolf, F., Goudsmit, J., Paul, D.A., Lange, J.M.A., Hooijkaas, C., Schellekens, P., Coutinho, R.A., Van der Noordaa, J., Risk of AIDS Related complex and AIDS in Homosexual Men with Persistent HIV-1 Antigenaemia. Brit. Med. J., 295:569-572, 1987.
19. Pedersen, C., Nielsen, C.N., Vestergaard, B.F., Gerstoft, J., Krogsgaard, K., Nielsen, J.O., Temporal Relation of Antigenaemia and Loss of Antibodies to Core Antigens to Development of Clinical Disease in HIV-1 Infection. Brit. Med. J., 295:587-589, 1987.
20. Newall, A.L., Malkovsky, M., Orr, D., Taylor-Robinson, D., and Dalgleish A.G. Antigen Test Versus Reverse Transcriptase Assay for Detecting HIV-1. Lancet 2:1146-1147, 1987.
21. Healey, D.S., Maskill, W.J., Neate, E.V., Beaton, F., Gust, I.D. A preliminary evaluation of five HIV antigen detection assays. J. Virol. Meth. 20:115-125, 1988.
22. Chaisson, R.E., Allain, J.P., Leuther, M., Volberding, P. Significant Changes in HIV-1 Antigen Level in the Serum of Patients Treated with Azidothymidine. New Eng. J. Med. 315:1810-1811, 1986.
23. Fischl, M.A., Richman, D.D., Grieco, M.H., Gottlieb, M.S., Volberding, P.A., Laskin, O.L., Leedom, J.M., Groopman, J.E., Mildvan, D., Schooley, R.T., Jackson, G.G., Durack, D.T., and King, D. The Efficacy of Azidothymidine (AZT) in the Treatment of Patients with AIDS and AIDS-Related Complex. New Eng. J. Med. 317:185-191, 1987.
24. Yarchoan, R., Perno, C.F., Thomas, R.V., Klecker, R.W., Allain, J.P., Wills, R.J., McAtee, N., Fischl, M.A., Dubinsky, R., McNeely, M.C., Mitsuya, H., Pluda, J.M., Lawley, T.J., Leuther, M., Safai, B., Collins, J.M., Myers, C.E., and Broder, S. Phase 1 Studies of 2',3'-Dideoxycytidine in Severe Human Immunodeficiency Virus Infection as a Single Agent and Alternating with Zidovudine (AZT). Lancet 1:78-80, 1988.
25. DeWolf, F., Lange, J.M.A, Goudsmit, J., Cload, P., DeGans, J., Schellekens, P.T.A., Coutinho, R.A., Fiddian, A.P., Van der Noordaa, J. Effect of Zidovudine on Serum Human Immunodeficiency Virus Antigen Levels in Symptom-free Subjects. Lancet 1:373-378, 1988.
26. Parks, W.P., Parks, E.S., Fischl, M.A. et al. HIV-1 Inhibition by Azidothymidine in a Concurrently Randomized Placebo-controlled Trial. JAIDS 1:125-130, 1988.
27. Chaisson, K.E., Leuther, M.O., Allain, J.P., et al. Effect of Zidovudine on Serum Human Immunodeficiency Virus Core Antigen Levels. Arch. Intern. Med. 148:2151–2153, 1988.
28. Acquired Immune Deficiency Syndrome (AIDS): Precautions for Clinical and Laboratory Staffs MMWR 31:577-580, 1982.
29. Sehulster, L.M., Hollinger, F.B., Dressman, G.R., and Melnick, J.L., Immunological and Biophysical Alteration of Hepatitis B Virus Antigens by Sodium Hypochlorite Disinfection. Appl. and Envir. Microbiol. 42:782-787, 1981.
30. Mahan, B.H., University Chemistry, Second Edition, Addison Wesley, p848, 1989.
31. Bond, W.W., Favero, M.S., Petersen, N.J., and Ebert, J.W., Inactivation of Hepatitis B Virus by Intermediate-to-High-Level Disinfectant Chemicals. J. Clin. Microbiol. 18:535-538, 1983.
32. U.S. Environmental Protection Agency, Office of Solid Waste, "Draft Manual for Infectious Waste Management", Washington, D.C., 1982. (USG PO 1982-381-082/325).
33. Merck Index, listing 4804, 1983.
34. Center for Disease Control. Update on acquired immune deficiency syndrome (AIDS). MMWR 31, 507-508, 1982.
35. Revision of the CDC Surveillance Case Definition for Acquired Immunodeficiency Syndrome, MMWR 1987;*36* (suppl no. 1S):1S-16S.
36. Kalbfleisch, J.D. and Prentice, R.L., The Statistical Analysis of Failure Time Data, John Wiley & Sons, 1980.

20