In-Vitro Screening for Viral Contamination of Blood Donations used in the Manufacture of Cutter Blood Products

1. Full details of the test procedures used for the testing of blood donations used in the manufacture of our products are included in the instruction leaflets supplied by the manufacturers of the test kits and reagents. These instructions are followed by our testing laboratory without any deviation.

   The following test procedures are used:-

   - ELISA Test Kit for Detection of Antibody to Human T-Lymphotropic Virus Type III (HTLVIII) (Electro-Nucleonics Inc, VIRGO Test)

   - Radioimmunoassay Test Kit for Detection of Hepatitis B Surface Antigen. (Electro-Nucleonics Inc, RIAUSURE II Test)

   - Reagent for the Determination of Serum Alanine Aminotransferase (ALT, GPT). (Electro-Nucleonics Inc, ALTAIRE Test Kit)

2. Quality control procedures carried out in relation to the assays are as described in the instruction leaflet for each kit.

3. The criteria by which donor units are excluded from processing are as follows:-

   3.1 Any units determined to be reactive by the VIRGO Elisa test for HTLVIII or the RIAUSURE II test for HB_sAg will be destroyed and the donor permanently deferred.

   3.2 If serum samples from any donor are positive by the ALTAIRE test (ie ALT levels are at least twice the upper limit of normal) the following procedure is used:

   - If the donor's ALT levels are between 2 and 5 times the upper limit of normal for the first time in 30 days and the plasma units are non-reactive, no action is taken.

   - If the donor's ALT levels are between 2 and 5 times the upper limit of normal for the second time in 30 days the unit is destroyed (even if it has tested non-reactive) and the donor is permanently deferred.

     If the second reactive is more than 30 days after the first no action is taken.

   - If the donor's ALT levels are more than 5 times the upper limit of normal for the first time the unit is destroyed and the donor permanently deferred.

Cutter Laboratories
Division of Miles Laboratories, Ltd.
Stoke Court, Stoke Poges
Slough SL2 4LY England
Phone: Farnham Common (02814) 5151
Telex: 848337

Early in [illegible]

In Vitro Sceening Tests for Viral Contamination of Blood Donations Used in the Manufacture of Blood Products

1) The following test procedures are used by CUTTER:-

- VIRGO ELISA TEST kit for Detection of Antibody to HTLV-III
  - Electro-Nucleonics Inc.
- RIAUSURE II kit for Detection of Hepatitis B Surface Antigen by Radioimmunoassay
  - Electro Nucleonics Inc.
- ALTAIRE kit for Determination of Serum Alanine Aminotransferase (ALT)
  - Electro Nucleonics Inc.

The manufacturer's instructions are followed by our testing laboratory; there are no deviations.

Copies of the package inserts for these kits are attached.

2) Positive and negative controls are carried out with each test unit as in the instructions ie with every 90 tests for antibodies to HTLV III (HIV) and every 600 for $HBsA_g$.

3) The criteria for exclusion of donors is detailed in our SOP entitled "Cutter System of Plasmapheresis". A copy of this is attached.

Marie Tatt

17.9.87

HUMAN T-LYMPHOTROPIC VIRUS [redacted]

VIRGO™ HTLV-III ELISA

Enzyme-Linked Immunosorbent [redacted]
Detection of Antibody to Hum[redacted]photropic Virus Type III
(HTLV-III)

HUMAN T-LYMPHOTROPIC VIRUS TYPE III

VIRGO™ HTLV-III ELISA

ELISA Test Kit for Detection of
Antibody to Human T-Lymphotropic
Virus Type III (HTLV-III)

INTENDED USE:

The VIRGO™ HTLV-III ELISA Test Kit is an Enzyme-Linked Immunosorbent Assay utilizing a purified preparation of Human T-Lymphotropic Virus Type-III (HTLV-III) as a substrate for the detection of circulating antibodies to HTLV-III in human serum or plasma.

SUMMARY:

The presence of circulating antibodies indicates exposure of an individual to an agent or its antigens and a subsequent immune response. An immune response is usually associated with prior infection and immunity to a particular disease, but examples exist such as Herpes Simplex Virus where an immune response as indicated by circulating antibodies does not impart protection. Recent epidemiological evidence indicates that an infectious agent transmitted through intimate contact, IV drug use or use of infected blood or blood products causes Acquired Immunodeficiency Syndrome (AIDS). This disease affects T-cell mediated immunity, resulting in severe lymphopenia and a reduced subpopulation of helper T-lymphocytes. Destruction of this T-lymphocyte population by the virus causes an immune deficiency, resulting in a reduced or deficient immune response to subsequent infections. Consequently infections become more severe and may cause death. At present there is no successful treatment for AIDS. The etiologic agent has been identified as a retrovirus, HTLV-III (1, 2, 3, 4, 5).

The implications of antibody to HTLV-III in an asymptomatic individual are not known. HTLV-III virus has been found in the presence of antibody to HTLV-III. The VIRGO HTLV-III ELISA was developed to detect antibody to HTLV-III so that potentially infectious units of blood and plasma could be eliminated from use in transfusion or manufacture into injectable products.

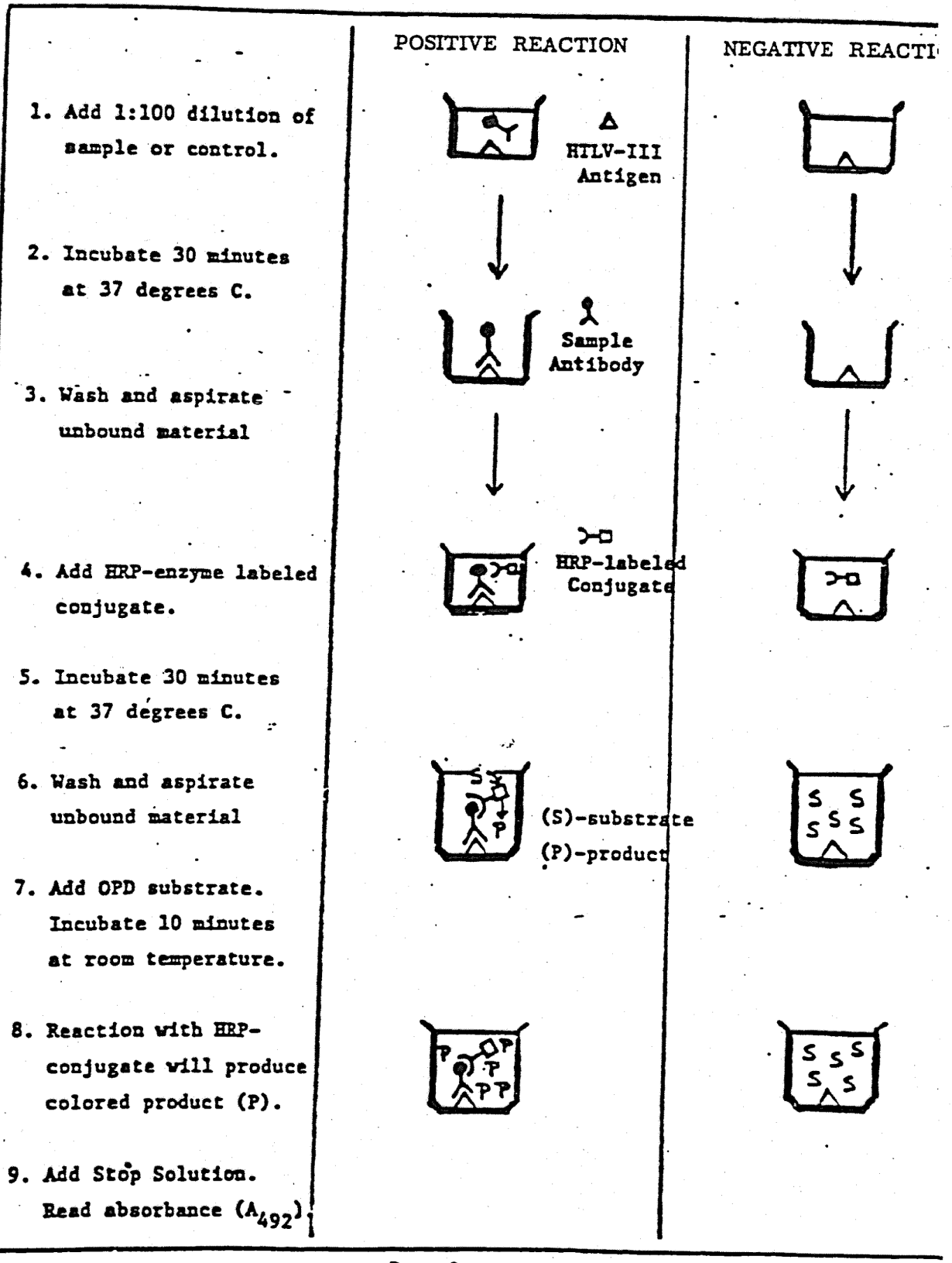
The ELISA procedure is a well established technique for the detection of antibody to viruses (6). The method is both highly sensitive and specific if adequately quality controlled. The 96 well microassay plate format lends itself to high throughput testing. The VIRGO™ HTLV-III ELISA kit is supplied with virus antigen coated microassay plates, control samples and all reagents necessary for detection of antibody to HTLV-III in human serum or plasma samples.

PRINCIPLE OF THE METHOD

Human T-Lymphotropic Virus Type III (HTLV-III) isolated in the laboratory of Dr. Robert Gallo (NCI) was propagated from a seed stock according to procedures established by M. Popovic (2). The cell line infected with HTLV-III is cultured and the culture supernatant is purified by centrifugation procedures. The viral concentrate is then inactivated in a two-step procedure using chemical and physical treatments.

Purified inactivated HTLV-III is adsorbed onto wells of a microassay plate. Serum or plasma samples diluted in a buffer are added to these wells. If antibodies specific for HTLV-III are present in the sample, they will form stable complexes with the HTLV-III antigens on the plate. A goat anti-human IgG (Heavy & Light chain specific) labeled with horseradish peroxidase is added. If the antigen/antibody complex is present, the peroxidase conjugate will bind and remain in the well. Enzyme substrate is then added. Color will develop in wells containing antibody. No color develops in negative wells. An acid stop solution is added to each well and the color read on a microassay plate reader at 492 nm. (See Diagram)

DIAGRAM OF PRINCIPLE



MATERIALS SUPPLIED

1. HTLV-III Microassay Plates: Plates containing 96 wells coated with inactivated, disrupted, purified Human T-Lymphotropic Virus Type III.

2. Anti-Human Conjugate: Anti-human IgG (Heavy & Light chain specific) (Goat) labeled with horseradish peroxidase (HRP) (lyophilized). Preservative: Thimerosal.

3. High Positive HTLV-III Serum Control: Inactivated human serum containing a high titer of antibody to HTLV-III (lyophilized). Preservative: Thimerosal.

4. Low Positive HTLV-III Serum Control: Inactivated human serum containing a low titer of antibody to HTLV-III (lyophilized). Preservative: Thimerosal.

5. Negative HTLV-III Serum Control: Human serum negative for antibody to HTLV-III (lyophilized). Preservative: Thimerosal.

6. Phosphate Buffered Saline. Diluent Buffer Component A. Preservative: Thimerosal.

7. Normal Goat Serum: Diluent Buffer Component B. Preservative: Thimerosal.

8. PBS/Tween 25X Concentrate. Phosphate buffered saline containing Tween 20. Preservative: Thimerosal.

9. OPD Substrate Tablets: O-phenylenediamine Dihydrochloride (OPD) chromogen.

10. OPD Substrate Buffer: Diluent for OPD Substrate Tablets

11. Stop Solution: 2N Sulfuric Acid (H_2SO_4)

VIRGO™ ELISA Test Kit for [redacted] of HTLV-III Antibody

List No. 4320 480 Determinations

Kit Contains:

5 HTLV-III Microtiter Plates (96 Wells each)

5 Vials (12.2ml) Conjugate Goat Anti-human IgG (H & L chain specific)

1 Vial (1.2 ml) Positive HTLV-III Control (Human)

2 Vials (1.2ml) Positive HTLV-III Control (Human)

1 Vial (1.2ml) Negative HTLV-III Control (Human)

5 Vials (88ml) Phosphate Buffered Saline

5 Vials (22ml) Goat Serum

2 Vials (40ml) Tween 25X Concentrate

1 Bottle (18ml) OPD Substrate

2 Vials (48ml) Substrate Buffer

1 Bottle (50ml) Solution

MATERIALS REQUIRED BUT NOT SUPPLIED

Non-absorbant bench cover
Test tubes and racks
Micropipettes; 50µl, 100µl,
Multipipettors; 50µl, 100µl and tips or automated dispenser.
Disposable pipettes; 1 ml, 5 ml, 10 ml
Ehrlenmeyer flasks; 100 ml, 500 ml, 1 and 4 Liter
Aluminum foil
Non-metallic forceps
Timer
Disposable gloves
Lab tissue
Safety pipetting bulb
Humidity chamber (Box or sealable bags containing moist towels)
37° C Dry incubator or
37° Humidified incubator (Plate sealers should be used)
Opaque plate cover
Deionized or distilled water
Microassay plate washer dispenser (opt.)
Single or dual wavelength spectrophotometer A492 with 600-620 nm as reference
Vacuum pump system
Autoclave
Refrigerator 2° to 8°C

PRECAUTIONS

1. The HTLV-III microassay plates were prepared from viral material inactivated by detergent and heat treatment. ENI has assayed for reverse transcriptase (RT) activity in inactivated preparations with negative results. Attempts at reisolation of the virus from samples of inactivated material inoculated into H9 cell line yielded no detectable live virus. However, good laboratory practice (GLP) dictates that these reagents be handled and disposed of as you would any potentially biohazardous material.

2. The positive control sera have been heated at 56°C for 1 hour a assayed for RT activity and reisolation of virus with negati results. This does not ensure the absence of viable HTLV-III viru and therefore these sera should be handled as potentiall biohazardous. Again, following GLP is imperative.

3. All human serum components have been tested by radioimmununoass and were found to be negative for hepatitis B surface antige (HBsAg). This does not ensure the absence of hepatitis B virus.

4. This kit contains acid. Do not combine acid waste with wast material containing sodium azide.

5. All materials used in this assay including reagents and sample should be disposed of in a manner that will inactivate huma hepatitis and HTLV-III virus.

   The preferred method for solid wastes (ie. plates, disposabl gloves, etc.) is autoclaving for 60 minutes at 121°C.

   The liquid waste may be decontaminated by addition of:

   Sodium Hypochlorite (bleach) at a 2.5 % concentration in the fina volume. The waste should be allowed to stand a minimum of 30 minute to inactivate the virus before disposal (7).

6. The components of this kit have been tested as a unit. Do no interchange components from other sources or from different lots o reagents.

7. Do not use reagents beyond their labeled expiration dates.

8. Do not handle OPD substrate tablets with fingers or permit contac with skin. Use nonmetallic forceps to handle tablets. Protect OPD tablets and prepared reagent from light.

9. All reagents must be at room temperature 15-30 minutes before use.

10. The HRP conjugate may be inactivated by water which has been deionized with polystyrene resin. Do not use this type of deionized water to make the Assay Wash Buffer.

11. If serum or plasma samples are stored, they should be kept at 2° to 8°C or frozen. Self-defrosting freezers are not recommended for this storage. Avoid multiple freeze-thaw procedures.

12. Do not use a water bath to incubate plates.

13. Avoid repeated opening and closing of the incubator during the conduct of the assay.

14. During 37° C incubation, evaporation must be prevented. Place plate in a covered box containing moistened paper towelling or cover plate with plate sealer if using a humidified incubator.

15. When using automated dispensors the tips must be adequately rinsed or replaced between each use.

16. For In Vitro Use Only.

Reagent Storage

1. Store unopened kit at 2° to 8° C upon receipt.

2. Stop solution is stored at room temperature.

3. PBS/Tween 25X concentrate and working dilution are stored at room temperature.
   Note: When concentrated PBS/Tween 25X is stored at 2° to 8° as in the unopened kit, crystals may form. Redissolve by warming at 37°C. Mix well before diluting.

4. Store reconstituted controls, diluent buffer, OPD Substrate Buffer and OPD Tablets at 2° to 8°C.
   Note:
   a. Reagents must be at room temperature 15-30 minutes before use.

   b. Bring OPD Tablet bottle to room temperature 15-30 minutes before opening. Return to 2°to 8°C after removal of required number of tablets. Do not remove desiccant from the OPD bottle.

REAGENT PREPARATION

1. Diluent Buffer: Component A-Phosphate Buffered Saline
Component B-Normal Goat Serum

Pour one vial Component B into one Component A vial. Mix thoroughly. Use within 5 days. Store at 2° to 8°C. Bring to room temperature 15 to 30 minutes before using.

2. High Positive/Low Positive/Negative Controls (lyophilized).
Reconstitute with 1.2 ml Diluent Buffer.
Mix thoroughly. Liquid controls are stable 5 days at 2° to 8°C.

3. Assay Wash Buffer. Dilute PBS/Tween 25X Concentrate with deionized or distilled water to final volumes listed below:

Note: When concentrated buffer is stored at 2°C to 8°C, crystals may form. Redissolve by warming 37°C. Mix well before diluting.

PBS/Tween 25X	Final Volume
16 ml	400 ml*
40 ml	1 liter

*400 ml is sufficient assay wash buffer for 1 plate.

Store at room temperature. Use within 5 days.

4. HRP Conjugate (lyophilized). Bring to room temperature 15-30 minutes before use. Reconstitute each HRP Conjugate vial with 12.5 ml Diluent Buffer. 12.5 ml is sufficient conjugate for 1 plate

Mix thoroughly. Use within 8 hours. Store unused conjugate at 2° to 8°C. Bring to room temperature 15-30 minutes before use.

5. HTLV-III Microassay Plates
Store at 2° to 8°C. Bring the required number of plates to room temperature 15-30 minutes before removing from its/their pouch(es).

6. OPD Substrate

Prepare reagent 15 minutes before use. Make sure that the bottle containing OPD tablets is at room temperature 15-30 minutes before opening. Each OPD tablet is sufficient for approximately 50 tests. Bring OPD Substrate Buffer to room temperature. In a dark or foil covered container, add 5 ml buffer for each tablet (See Chart). Mix well before using. Use prepared OPD Reagent within 60 minutes.

No. Plates	No. OPD Tablets	ml OPD Substrate Buffer
1	3	15
2	5	25
3	7	35
4	9	45
5	11	55

NOTE: Avoid metal contact with OPD tablets or substrate solution. Protect solution and tablets from light. It is essential that clean labware be used for preparation of the OPD Substrate. Trace metal ions or other contaminants may interfere with results.

SPECIMEN COLLECTION AND HANDLING

1. Handle all blood, plasma and serum as if capable of transmitting hepatitis and/or HTLV-III viruses.

2. Optimal performance of ELISA depends upon fresh serum or plasma samples (clear, non-hemolyzed). Specimens should be collected aseptically. Early separation from the clot prevents hemolysis of serum. Heat inactivated serum or plasma is not recommended.

3. If samples are extremely lipemic, icteric or bacteriologically contaminated, preferably another sample should be drawn. If another is not available, filtration (0.45µ) or centrifugation (approximately 3000 X G for 10 minutes may be used to improve sample quality.)

4. Avoid multiple freeze-thaw cycles which may result in sample deterioration and cause erroneous results. Self-defrosting freezers are not recommended for storage of samples.

5. Undiluted specimens may be stored for up to seven days at 2° to 8°C if they are suitably vialed and stoppered. For longer storage, samples should be frozen at -20° C or colder. Place at 37° C only until the samples are thawed. Remove and mix thoroughly before use.

6. Store 1:100 sample dilution at 2° to 8°C. Use within 8 hours of preparation. Bring to room temperature 15-30 minutes before using.

TEST PROCEDURE

NOTE: Specimens may contain infectious HTLV-III or hepatitis virus. Handle as if capable of transmitting virus. Plasma or serum samples should be screened at a standard 1:100 dilution on the HTLV-III microassay plate. Initial reactives must be retested in duplicate on an HTLV-III microassay plate.

1. Into clean test tubes, or other comparable container, dilute 10 µl of each sample to be tested with 990 - 1000 µl working Diluent Buffer and mix well. Alternatively, two serial 1:10 dilutions may be performed to yield 1:100. Both methods may be performed with an automatic diluting device capable of performing one or both of the methods of dilution.

2. Remove the required number of microassay plates from the refrigerator and allow them to come to ambient temperature (approximately 15to 30 minutes). Remove the plates from their pouches just before use and label.

3. Dispense 100 µl of each diluted sample or reconstituted control samples (do not dilute controls) into the respective wells of the microassay plate being used. Use a clean pipette for each sample.

4. It is necessary to have 2 wells with High Positive HTLV-III Control, 3 wells with Low Positive HTLV-III Control, 2 wells with Negative HTLV-III Control and 1 well with Diluent Buffer reagent only on each microassay plate used. The best quality control of the procedure is assured if controls provided are added just prior to incubation.

NOTE: A suggested plate format for controls is illustrated below. The microassay plate reader in your laboratory may dictate that another format be used.

5. In a dry incubator place the plates in a prewarmed box with dampened towels. If a humidified 37° C chamber is used, the plates should be covered with a plate sealer. Alternatively in an humidified incubator the plates may be placed in a ''Ziploc'' bag. Do not stack more than 2 plates. Incubate for for 30 ±0.5 minutes.

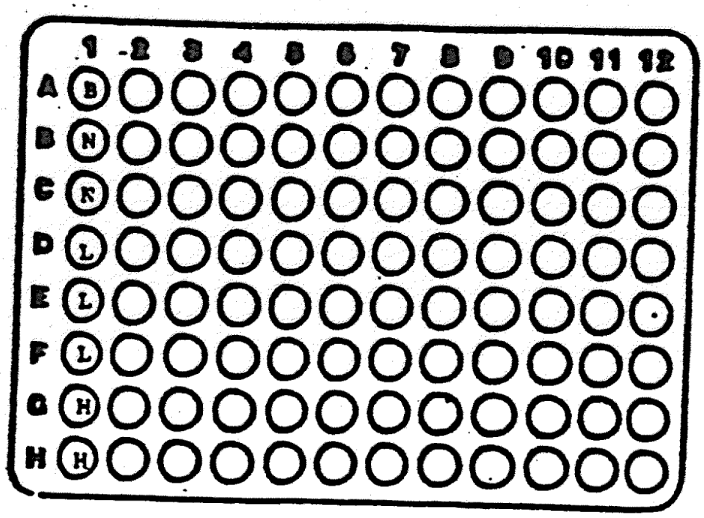
[Ziploc® is the registered trademark of the Dow Chemical Co. Indianapolis, Ind.] (In Print use as footnote)

6. Aspirate and wash each plate five times with the 250 to 300 µl/well Assay Wash Buffer. Automated washers should be adjusted to fill each well completely without overfilling. After the final wash, be sure all of the buffer is removed from each well. Sharply tap the plate upside down on towelling to remove the last remaining liquid. NOTE: Proper wash procedure is essential for good assay performance.

7. Dispense 100 µl of rehydrated HRP Conjugate into each well of the microassay plate.

8. Incubate the conjugate-filled plate for 30 ± 0.5 minutes at 37°C in a prewarmed humidified chamber.

9. Prepare OPD substrate 15 minutes before use in step 10.



B - Diluent Blank (For blanking plate reader)

N - Negative Control

L - Low Positive Control

H - High Positive Control

10. Aspirate and wash each plate five times with 250 to 300 µl/well Assay Wash Buffer. After the final wash, be sure all buffer is removed from each well as in step 6.

11. Add 100 µl of freshly prepared OPD Substrate reagent into each well.

12. Incubate the OPD Substrate filled plates for 10 minutes at room temperature (15 to 25 °C). Start the timing with the addition of the reagent to the first well. Use an opaque cover to protect from light.

13. Stop the reaction by adding 50 µl Stop Solution to each well in the same order used in the addition of the OPD substrate reagent.

14. Ten minutes after adding the Stop Solution, read the developed color in each well on a plate reader at 492 nm. Test must be read within one hour.

NOTE: The reader should be blanked on the Diluent Buffer well. Bichromatic absorbance measurements with a reference wavelength of 600 - 620 nm is recommended when available.

15. Record the absorbance results on the data sheets provided. Include the kit master lot number, date, operator name and any notes about the run. If a hard copy of the absorbance readings is provided it may be attached to the data sheets.

RESULTS

VALIDATION OF TEST PERFORMANCE

The control results must be examined before the sample results can be interpreted.

1. Determine the mean absorbance of the control values: Negative (N), Low Positive (LP), High Positive (HP) See example below.

N	LP	HP
0.023	0.187	0.822
0.017	0.201	0.867
-	0.199	-
0.040	0.587	1.689

$\overline{NX}$ = 0.040/2 = 0.02

$\overline{LPX}$ = 0.587/3 = 0.20

$\overline{HPX}$ = 1.689/2 = 0.84

Acceptable Range

$\overline{NX}$: $\leq$ 0.050

$\overline{LPX}$: 0.100 – 0.300

$\overline{HPX}$: $\geq$ 0.500

An individual control value is out of range if it deviates by more than 30% from its control mean. If only one individual control value is out of range, but the mean of each control falls within the acceptable range, the run is considered valid.

See below for calculations of out of range values.

If 2 or more control values each deviate by more than 30 % from their respective control means, the run is invalid and should be repeated.

OUT OF RANGE VALUES

A single control value must be excluded if it deviates by more than 30 % from its control mean.

Example: Low Positive Control

1. Determine Mean:

0.070
0.120
0.140
0.330

X = 0.330/3 = 0.110

2. Subtract out of range value from mean:

0.110 - 0.070 = 0.040

3. Determine percent away from mean:

$\frac{0.040}{0.110}$ X 100 = 36%

INTERPRETATION OF RESULTS

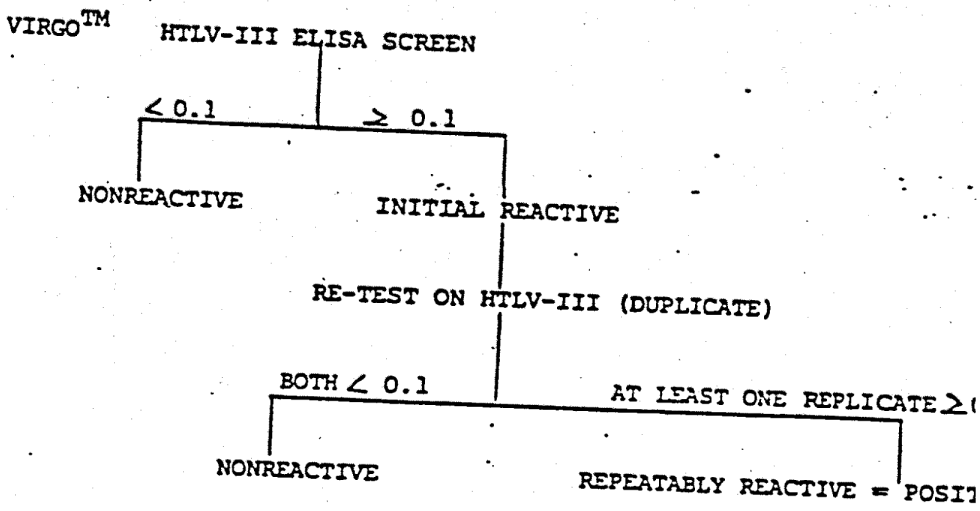
1. A cut off value of 0.100 absorbance units (A492) has been established.

2. If the initial test result of a sample is $\geq$ 0.100, retest in duplicate on an HTLV-III microassay plate using the original sample source.

3. If both retest values are < 0.100, the repeat test is nonreactive and the interpretation of the testing is nonreactive for HTLV-III antibodies.

   If both retest values are $\geq$ 0.100 or if one of the duplicates is <0.100 and one is $\geq$ 0.100, the interpretation of the testing result is repeatably reactive and the sample should be considered positive for HTLV-III antibodies by the criteria of the VIRGO HTLV-III ELISA test.

SUMMARY



EXPECTED VALUES

Table 1 illustrates the typical performance in 4 clinical studies

TABLE 1

For fresh plasma/serum samples (Sites A, B, C) the reactivity rate in random donors for the repeat VIRGO™ HTLV-III ELISA is 0.7 %. Test results at Site D represent a freezer study of sera drawn from random individuals and stored for varying lengths of time. A portion of the samples were drawn in 1974, prior to the onset of AIDS as a disease state. As stated above repeated freezing and thawing or heat inactivation of serum or plasma can result in elevated absorbances in the ELISA test which are not related to specific antibodies. Researchers can expect a higher than average rate of reactivity for samples which have been repeatedly frozen and thawed.

Each institution should establish its own reactivity rate.

TABLE 2

A summary of the VIRGO™ HTLV-III ELISA results in patient populations (Table 2) shows the reactivity of the test for various disease states.

PERFORMANCE CHARACTERISTICS OF THE TEST

At present there is no recognized standard for establishing the presence and absence of HTLV-III antibody in human blood. We are, therefore, computing sensitivity based on the clinical diagnosis of AIDS and specificity based on random blood and plasma donors. Our studies show that:

1. Sensitivity based on an assumed 100% prevalence of HTLV-III antibody in AIDS patients is estimated to be 99.6%.

2. Specificity based on an assumed zero prevalence of HTLV-III antibody in random plasma and blood donors is estimated to be 99.2%.

LIMITATIONS OF THE PROCEDURE

1. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, sample and reagent pipeting, careful washing and timing of incubation steps are essential for accurate, reproducible detection of HTLV-III antibody by the VIRGO HTLV-III ELISA Assay.

2. Use fresh serum or plasma samples or once frozen samples thawed at 37°C. Sample degradation as well as multiple freeze-thaw cycles may cause spurious results.

3. The primary use of the HTLV-III antibody test is to screen blood and plasma donations so that units containing antibody can be identified and eliminated or restricted to further manufacturing into non-injectable products. It is inappropriate to use this test as a screen for AIDS, or as a screen for members of groups at increased risk for AIDS in the general population. The presence of HTLV-III antibody is NOT a diagnosis of AIDS. Individual blood/plasma donors who are determined to be repeatably reactive should be referred for medical evaluation which may include additional testing, such as antibody detection by the Western Blot technique, if that has not been done at the collecting facility.

4. A non reactive test result does not exclude the possibility of exposure to or infection with HTLV-III.

5. Falsely reactive test results can be expected with a test kit of this nature. The proportion of reactives that are falsely reactive will depend on the sensitivity and specificity of the test kit and on the prevalence of HTLV-III antibody in the population to be screened.

   The prevalence of HTLV-III antibody in random donors is not known, but the higher the prevalence of HTLV-III antibody in a population, the lower the proportion of falsely reactive samples. With the estimated sensitivity and specificity of the VIRGO test kit and an assumed prevalence rate of 0.1%, out of every 100 repeatably reactive samples, 89 will not contain HTLV-III antibody. If the HTLV-III prevalence rate is 1.0%, then out of every 100 repeatably reactive samples, there will be only 44 that do not contain HTLV-III antibody.

6. Samples which are considered positive after testing as described above should be retested to establish whether or not they are repeatably positive. The retest should be performed using the original sample source.

TABLE 1

ELISA RESULTS FOR RANDOM BLOOD AND PLASMA DONOR POPULATIONS

DONOR CENTER	NO. SAMPLES	INITIAL TEST	PERCENT TOTAL	REPEAT TEST	PERCENT TOTAL
A *	4918	36	0.7	15	0.3
B	5809	117	2.0	63	1.1
C **	4487	38	0.8	28	0.6
D	1177	50	4.2	21	1.8
Totals	16391	241	1.47	127	0.77

* Samples from sites B & D were serum, other sites were plasma.

** Samples from site D were from a freezer study of stored random donor specimens. Some of the samples have been stored for up to 10 years. The higher incidence of reactive specimens is due to conditions of storage.

All samples in these studies were from random blood and plasmapheresis donors.

Sites A, B and C are test centers for plasmapheresis donors. Site D is a volunteer blood bank.

TABLE 2

ELISA RESULTS FOR DIFFERENT PATIENT POPULATIONS

DIAGNOSIS	NO. SAMPLES	INITIAL REACTIVE	PERCENT TOTAL	REPEAT REACTIVE	PERCENT TOTAL
AIDS/KS/OI	236	235	99.6	235	99.6
ARC	139	128	92.1	127	91.4
AIDS CONTACTS	3	2	66.7	2	66.7
HEMOPHILIACS	469	124	26.4	113	24.1
HHS MALES	114	68	59.6	66	57.9
OTHER DISEASES	382	18	4.7	13	3.4

AIDS - Acquired Immunodeficiency Syndrome
KS - Kaposi's Sarcoma
OI - Opportunistic Infection
ARC - AIDS Related Complex
HHS - Healthy Homosexual males

Other Diseases include: Rheumatoid Arthritis, Leukemias, Lymphomas, Myelomas, Melanoma, non-AIDS Kaposi's Sarcoma, Hepatitis B Virus and Bone Marrow Transplant recipients.

The specimens represented in this table were collected from 2 clinical research sites, and from a community blood bank engaged in research on various diseases related to blood.

The percent reactive samples for hemophiliacs is lower than current results. A portion of these specimens were collected before the onset of AIDS in the United States i.e., prior to 1978.

BIBLIOGRAPHY

1. Barre-Sinoussi, F., J. C. Chermann, F. Rey et al. 1983. Isolation of a T-Lymphotropic Retrovirus from a patient at risk for Acquired Immune Deficiency Syndrome. Science 220:868-871.

2. Popovic, M., M. G. Sarngadharan, E. Read, and R. C. Gallo. 1984. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and Pre-AIDS. Science 224:497-500.

3. Gallo, R. C., S. Z. Salahuddin, M. Popovic, et al. 1984. Frequent detection and isolation of cytopathic retrovirus (HTLV-III) from patients with AIDS and at risk for AIDS. Science 224:500-503.

4. Schupbach, J., M. Popovic, R. V. Gilden, et al. 1984. Serological analysis of a subgroup of human T-lymphotropic retroviruses (HTLV-III) associated with AIDS. Science 224:503-505.

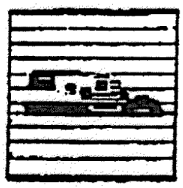
5. Sarngadharan, M. G., M. Popovic, L. Bruch, et al. 1984. Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS. Science 224:506-508.

6. Voller, A., D. E. Bidwell and A. Bartlett. 1979. ELISA in Infectious Diseases P. 51-53. In ''The Enzyme Linked Immunosorbent Assay (ELISA).'' Sponsored by Dynatech Europe, Borough House Rue du Pre, Guernsey, G.B.

7. Spire, D., L. Montagnier, F. Barre-Sonoussi and J. C. Chermann. 1984. Inactivation of Lymphadenopathy Associated Virus by Chemical Disinfectants. Lancet. Oct. 20, 899-901.

TENTATIVE

EM Diagnostic Systems, Inc.



EPOS SysteMate

Diagnostic Reagents

ALT 67209

INTENDED USE

For the quantitative determination of Alanine Transaminase in serum or plasma.

SUMMARY AND PRINCIPLE

In 1956, Wroblewski et al.[1] investigated alanine amino transferase (ALT), formerly known as glutamic pyruvic transaminase (GPT), activity in human serum and introduced a spectrophotometric assay based on the measurement of NADH consumption. The following method is based on the principles outlined by Wroblewski and La Due but is modified according to the IFCC recommendations[2]. Substrate concentrations are optimized. Tris replaces phosphate as a buffer and LDH has been added to remove endogenous pyruvate in the serum.

Serum is preincubated with the buffer-substrate reagent containing LDH to remove endogenous pyruvate. The reaction for alanine amino transferase is started by the addition of 2-oxoglutarate. The enzyme ALT transfers the amino group from alanine to 2-oxoglutarate to form pyruvate and glutamate. The pyruvate enters a lactate dehydrogenase catalyzed reaction with NADH to produce lactate and NAD^+. The decrease in absorbance due to consumption of NADH is measured spectrophotometrically.

$$\text{2-Oxoglutarate} + \text{L-Alanine} \xrightleftharpoons{\text{ALT}} \text{Glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightleftharpoons{\text{LDH}} \text{Lactate} + \text{NAD}^+$$

In the EPOS 6-point kinetic method, the rate of NADH decrease is determined spectrophotometrically at 334 nm and is directly proportional to the ALT activity in the sample. Premature substrate depletion is detected by the Start Kinetic Option and high pyruvate levels are detected by the Interference Option of the EPOS System.

REAGENTS

FOR IN VITRO DIAGNOSTIC USE

67209A ALT Buffer, 7 × 30 ml

L-Alanine	600 mmol/L
Tris HCl	115 mmol/L
pH	7.5 @ 30°C

WARNING! CONTAINS SODIUM AZIDE! Sodium Azide may react with copper and lead plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

67209B ALT Substrate, 7 × 30 ml

NADH	6.5 µmol/vial
Lactate Dehydrogenase	72 U/vial

67209C ALT Starter, 1 × 25 ml

2-oxoglutarate	180 mmol/L
Tris HCl	65.4 mmol/L

WARNING! CONTAINS SODIUM AZIDE! Sodium Azide may react with copper and lead plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

Preparation of Reagents

Prepare Working Reagent R_1 by reconstituting ALT Substrate (B) with entire contents of ALT Buffer (A). Swirl to dissolve and allow to stand 10 minutes. Transfer appropriate amount to the R_1 reagent vessel.

ALT Starter (C), R_2, is ready for use. Transfer appropriate amount to R_2 reagent vessel.

Storage Instructions

Store SysteMate ALT Set as packaged at 2-8°C. Storage must not exce the expiration date on box label. The reconstituted Reagent is stable fo weeks at 2-8°C and 6 days at 15-25°C. The Starter is stable up expiration date at 2-8°C and 4 weeks at 15-25°C. Care should be taken minimize evaporation if the Working Reagent is stored in the R_1 or vessels.

Indications of Deterioration

Clumping, insolubility or discoloration of the normally white, dry Reage indicates decomposition. Any evidence of microbial growth in either t reconstituted Reagent, Buffer or Starter warrants discontinuance of us

SPECIMEN COLLECTION AND PREPARATION

Use clear, unhemolyzed serum that has been separated from the cells soon after collection as possible. It is best to measure the enzyme activ within a few hours of collecting. The serum loses less than 10% of activity within 4 days when stored at 2-8°C, and within 3 days when stor at 15-25°C.[3,4]

Perform the ALT determination as soon after collection as possible.

Interfering Substances

The results of studies at EM Diagnostic Systems laboratories show th the following substances interfere with ALT determinations:

1. Method dependent
   - –GLDH interferes in the presence of pathologically high ammoniu ion concentrations
   - –pyruvate in high concentrations interferes with the determination t consuming NADH (Remedy: Dilution)
2. Method independent
   - –increased ALT activity through anabolics, androgens, antibiotic contraceptives, opiates, phenothiazines

For further information on interfering substances, refer to the publicatic by Young, et al. *Clin. Chem. 21:* No. 5, ID-432D, 1975.

PROCEDURE

Reagents Provided

ALT Buffer (A)
ALT Substrate (B)
ALT Starter (C)

Materials Required But Not Provided

EPOS Analyzer
EPOS Sample Cups
EPOS Reagent Vessels (R_1 and R_2)

Basic Analytical Parameters of Method

Reagent 1 Volume	250 µl
Reagent 2 Volume	25 µl
Sample Volume	25 µl
Wavelength	334 nm
Temperature	30°C

Procedural Outline

NOTE: Operator should refer to the EPOS Operations Manual for mor detailed information, and become thoroughly familiar with its operatio prior to performing any assays.

1. Load patient samples into sample cups and place in sample chain.
2. Load R_1 and R_2 into appropriate positions on Analyzer. (Make sur

appropriate controls or standards are loaded into the appropriate wells on the R_1 vessel lid).
3. Enter "STA" (Start) code into Data Terminal.
4. Select and enter test method (either by method name, method number or appropriate softkey).
5. Select and enter sample range to be analyzed.
6. The Data Terminal will automatically transfer those patient (sample) numbers to be analyzed to the EPOS Analyzer.
7. The EPOS Analyzer will automatically call up the chosen method and corresponding parameters.
8. Once the Analyzer signals ready, press "Start" on the Analyzer.
9. Those patients to be analyzed will automatically be selected by the EPOS Analyzer.

Results and Calculations

Results in U/L will be obtained directly from the Analyzer or selectively by patient via the Data Terminal.

Quality Control

The regular use of control serum is advised to evaluate day-to-day consistency. Good technique dictates that controls with normal and abnormal values be used.

LIMITATIONS OF PROCEDURE

Refer to Interfering Substances.

EXPECTED VALUES

Expected values may vary with age, sex, diet and geographical location. Good practice dictates that each laboratory determine its own expected values.

Expected values as reported by Tietz[4] are:

Males:	6-21 U/L at 30°C
Females:	4-17 U/L at 30°C

SPECIFIC PERFORMANCE CHARACTERISTICS

The following data were obtained using the exact procedure described in this insert. All reagents were manufactured as for sale.

Accuracy: Various dilutions of pooled elevated human sera were run in duplicate. The results were linear to 250 U/L.

Sensitivity: Using the exact procedure outlined on the EPOS Analyzer, the change in absorbance per minute for 1 U/L of ALT is 0.0005 (may vary slightly with reconstitution of lot).

BIBLIOGRAPHY

1. Wroblewski, F. and LaDue, J.S., *Proc. Soc. Exp. Biol. Med. 91*:569, (1956).
2. Clin. Chem. Acta 105, 147F-172F *J. Clin. Chem. Clin. Biochem. 18*:521-534, (1980).
3. Bergmeyer, H.U., *Clin. Chem. 18*:1305, (1972).
4. Engelhardt, A. and Noelges, A., *Arzt. Lab. 16*:42, (1970).
5. Tietz, N.W. (ed.), *Fundamentals of Clinical Chemistry*, Philadelphia: W. B. Saunders, Company, 1982, p. 1208.

†Trademark of Bio-Dynamics/BMC
††Trademark of Gilford Electronics, Inc.

TECH HOT LINE

If you need any technical assistance regarding our products, please call: 1-800-257-5541 in New Jersey: 800-232-6533

EM Diagnostic Systems, Inc.
480 Democrat Road
Gibbstown, NJ 08027
(609) 423-6300
(800) 257-5541
(In NJ 800-232-6533)

Issued 9/84
R9888

ALTAIRE™

ELECTRO-NUCLEONICS, INC.

ALT$_{LTS}$

PACKA
INSE

REAGENT FOR THE DETERMINATION OF SERUM ALANINE AMINOTRANSFERASE (ALT,GPT)

INTENDED USE

This procedure is intended for the quantitative determination of serum alanine aminotransferase activity using the ALTAIRE™ Chemistry System.

This kit contains either 10 x 30.0 mL vials (RA 10-60) or 20 x 15.0 mL vials (RA 20-60) of ALTAIRE™ ALT$_{LTS}$ Reagent.

TEST SUMMARY AND EXPLANATION

The enzyme alanine aminotransferase is clinically significant in the diagnosis of hepatic disorders.[1] This enzyme catalyzes the transfer of an amino group from L-alanine to 2-oxoglutarate with the formation of pyruvic and glutamic acids. Early colorimetric methods were based on measuring the amount of pyruvate formed after its conversion to pyruvate hydrazone.[2] The ALTAIRE ALT$_{LTS}$ Assay is based on Henry's[3] modification of the method of Wroblewski and LaDue.[4]

In this procedure, the pyruvate formed reacts with reduced nicotinamide adenine dinucleotide (NADH) in the presence of lactate dehydrogenase (LDH) to form lactic acid and oxidized nicotinamide adenine dinucleotide (NAD).

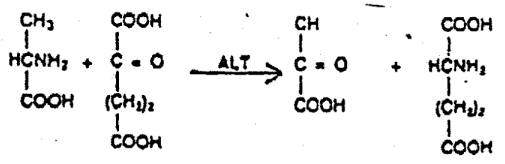
CLINICAL SIGNIFICANCE[1]

The activity of alanine aminotransferase (ALT,GPT) in serum is clinically useful in the differentiation of hepatic disorders, especially when its activity is compared to that of aspartate aminotransferase (AST,GOT).

In cases of hepatic necrosis, the activity of serum ALT is typically greater than that of AST; whereas in cirrhosis and metastatic carcinoma the reverse often occurs. Cases of myocardial infarction, however, show ALT levels within the reference interval, while the AST may frequently be 20-30 times higher.

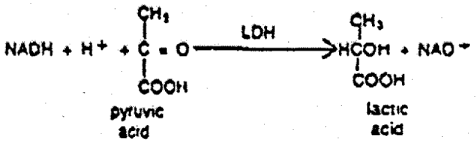
PRINCIPLE OF THE PROCEDURE

The alanine aminotransferase in serum converts the L-alanine acid and 2-oxoglutarate in the reagent to pyruvic and glutamic acid:



The rate at which this reaction proceeds is determined by the rate at which pyruvic acid is formed, and is a measure of the ALT (GPT) activity in the serum.

In the presence of excess LDH (which acts as an auxiliary enzyme) NADH (a co-enzyme), the pyruvic acid is converted to lactic acid, and NADH oxidized to NAD:



The rate of conversion of NADH to NAD$^+$ can be determined by observing the decrease in absorbance at 340 nm after a lag phase. This occurs because NADH absorbs strongly at this wavelength whereas NAD$^+$ does not. As can be seen, this rate of conversion from NADH to NAD is directly related to the activity of ALT (GPT).

The activity of ALT (GPT) in serum is expressed as International Units per liter (U/L), where one International Unit of activity represents the formation of one micromole of NAD$^+$ per minute under the assay conditions used.

REAGENT CONTENTS

When properly dissolved as directed below, the ALTAIRE ALT Reagent has the following composition:

ACTIVE INGREDIENTS	CONCENTRATION
Buffer, pH 8.0 (25°C)	100 mmol/L
L-Alanine	500 mmol/L
2-Oxoglutarate	15 mmol/L
Nicotinamide adenine dinucleotide (reduced) (NADH)	0.22 mmol/L
Lactate dehydrogenase	≥ 900 U/L

PRECAUTIONS

For In Vitro Diagnostic Use

The toxicological properties of this reagent have not been determined. Avoid ingestion.

MATERIALS REQUIRED

1. ALTAIRE™ Chemistry System and accessories
2. ALTAIRE™ ALT$_{LTS}$ Reagent
3. Distilled water
4. Pipettes capable of delivering 15.0 mL or 30.0 mL
5. Sample tubes
6. Bottle for Working Reagent

REAGENT PREPARATION

A. Kit RA 10-60 (10 x 30.0 mL vials)
Dissolve the contents of one vial of ALTAIRE ALT$_{LTS}$ Reagent in 30.0 mL distilled water. Dissolve by gentle but continuous inversion of the vial. DO NOT SHAKE.

B. Kit RA 20-60 (2
Dissolve the co
15.0 mL distille
the vial. DO NO

Prepare sufficie

REAGENT STORA

1. When stored
stable until the
2. The dissolved
(2°-8°C).
3. Should the dis
340 nm, discar
4. Should the dry
tamination is

SPECIMEN COL

1. Unhemolyzed
2. ALT activity is
refrigerated at

CHEMISTRY PA

ANALYSIS: ALT	
CODE	#3
TEMP.	37°
SAMPLE:	
BLANK	NO
VOLUME (N)	15.0 µL
VOLUME (R)	5.0 µL
DISP. TIME	−10 10"
REAGENT 1	
PUMP #0,	265 µL
	275 µL
	7
	−200"
REAGENT 2	
PUMP #1,	25 µL
	25 µL
PUMP #1,	19
PUMP #1,	−10 25"
WAVELENGTH	340nm
FILTER FAC	1.025
DATA REDUCT.	ZERO ORDER (S)
RMS, MAX.	1.000 mA
Ø MIN.	.7500 A
Ø MAX.	2.000
CALCULATI	#0
SP (K_2)	− 3059 E-3
UNITS	U/L
ATT. LEV.,	1.000 U/L
	850.0 U/L
† REF. LEV.	7.000 U/L
	33.00 U/L
†† CTR. (NORM)	/20
(ABNO	/20

†R is recomme determine the reference interval for its particula

††The number of running control sera should be determined by

PRESENTATIO

1. EVALUATE a batch summary and statistical report for all values. Allows for acceptance or rejection of the or all results from selected controls and unknown sa
   a. In this m by the date and time, a system number

b. The calculated values for the controls and unknown sam then printed in the order of analysis.
c. Rerun samples are designated by an asterisk.
d. Anomalous results are indicated by a non-numeric printed in place of a result. For a complete explanation messages, refer to Section 3 of the ALTAIRE Operating
e. The last part of the printout consists of the calculated stat controls and patients.

2. REPORT MODE—Once the results are evaluated and acce tient results can be printed in the REPORT MODE using (both) of two format styles:
   a. A complete set of patient reports printed out in the same they were entered (or processed).
   b. A single report showing all accepted chemistries perform individual patient as selected by the operator according test number.

CALCULATION

CALCULATION—Performed automatically by the AL microprocessor using the following calculation model #0.

$$R = (K_1 + K_2) \times Conc$$

For this assay:

R = Δ A/time (slope of the sample reaction)
K_1 = 0 (not applicable to this assay)
K_2 = $\frac{1}{KT\ Factor}$
Conc. = U/L

Therefore:

$$\Delta A/min = (0 + \frac{1}{KT}) \times U/L$$

or

$$KT \times \Delta A/min = U/L$$

When using the zero order (slope) data reduction model for an the absorbance change at 340nm is monitored during the assay calculated slope (ΔA per minute) is multiplied by a factor (KT below) to obtain the ALT activity of the sample in International liter at the programmed temperature.

FACTOR DERIVATION

The activity of ALT in serum is expressed as International Units One International Unit of ALT activity represents the formatio micromole of NADH per minute under the assay conditions us

$$U/L = \frac{\Delta A/min \times 10^3 \times 0.305 \times 10^3}{6.22 \times 10^3 \times 0.015 \times 1}$$

where

Δ A/min	= absorbance change per minute
10^3	= conversion of mL to L
0.305	= reaction volume in mL
10^3	= conversion of millimoles to micromoles
6.22×10^3	= molar absorptivity of NADH
0.015	= sample volume in mL
1	= light path in cm

Therefore:

U/L = Δ A/min x 3289

Since this is a decreasing reaction and since the calculation this assay requires the reciprocal $\frac{1}{(KT)}$ of this factor, 0.00030 programmed as − .3059 E-3.

CALIBRATION

Calibration is performed automatically by the A microprocessor.

QUALITY CONTROL

It is good practice to assay both normal and abnormal control sera on a regular basis. The ALTAIRE ALT_{LTS} Assay is an enzymatic procedure with values given in International Units at the programmed temperature. When using commercial control sera, the consistency of values from run to run is more important than the actual value obtained.

NOTES AND LIMITATIONS

1. If the sample has an ALT activity higher than 650 U/L, a message is printed in place of the result. Such samples should be rerun. Refer to ALTAIRE™ Operating Manual for the Rerun Procedure.
2. Erythrocytes contain 3-5 times more ALT(GPT) than serum, therefore only minimal hemolysis can be tolerated.
3. Certain drugs may interfere with the assay or affect the circulating level of ALT(GPT). For a complete listing of such drugs, see Young, et al.[3]
4. The performance of the ALTAIRE ALT_{LTS} Assay has been verified at 37°C

EXPECTED REFERENCE INTERVAL at 37°C

Serum samples from 99 asymptomatic adults were analyzed for ALT activity using the ALTAIRE ALT_{LTS} Reagent Kit. The following results were obtained:

Mean (U/L)	20.2
SD	6.6
Reference Interval (U/L) (Parametric)	7 - 33

IT IS RECOMMENDED THAT EACH LABORATORY DETERMINE THE REFERENCE INTERVAL FOR ITS PARTICULAR POPULATION.

PERFORMANCE CHARACTERISTICS – at 37°C

A. REPRODUCIBILITY – determined on the ALTAIRE™ Syste ALT levels. The three levels were assayed at least 5 times run, two runs per day, for a total of 20 runs.

The pooled within-run precision was found to be:

Mean (U/L)	26	98
SD	1.1	1.3
CV, %	4.2	1.3
n	178	178

The total assay precision was found to be.

Mean (U/L)	26	98
SD	1.3	1.5
CV, %	5.2	1.5
n	178	178

B. PATIENT CORRELATION – A series of 50 serum specimen values ranging from 3-547 U/L were assayed by both the A System (y) and the FLEXIGEM™ System (x). Least-squares r analysis of the data gave a correlation coefficient of 0.99. linear relationship expressed as: $y = 0.989x + 10.2$

REFERENCES

1. *Clinical Diagnosis*, 15th ed., J. Davidson and J.B. Henry, E Saunders Co., Philadelphia, PA, 1974.
2. *Clinical Chemistry*, 2nd ed., R.J. Henry, et al., Eds., Harper New York, NY, 1974.
3. Wroblewski, F., and LaDue, J.S., *Proc. Soc. Exp. Biol. Me* 4. (1956).
   Young, D.S., et al., *Clin. Chem.* 21, 242D-244D (1975).

ALTAIRE™ ALT_{LTS}
REORDER NO. RA 10-60 (10 x 30.0 mL vials) REV.
RA 20-60 (20 x 15.0 mL vials) P/N 90

Manufactured by
ELECTRO-NUCLEONICS, INC. 350 PASSAIC AVE., FAIRFIELD, N