HTLV 1+2 Ab
ELISA TEST SYSTEM

REACTIVA SEARCH

INTENDED USE

The Reactiva Search.'s Human T-cell Lymphotropic Viruses types I (HTLV I) and II (HTLV II) are closely related human retroviruses, showing a high degree of homology. HTLV I is pathogenically associated with adult T cell leukaemia and with a chronic neurological disorder. In contrast, HTLV II, which was first isolated from a patient with a variant of hairy cell leukaemia, has not been associated with any specific disease. While HTLV I infection is endemic in south-western Japan, the Caribbean and some regions of Africa, HTLV II has been reported mainly in intravenous drug abusers. Infection generates a strong immunological response in the first stage and antibodies to HTLV I and II may be detected in infected patients. Enzyme Immunoassays have been developed recently for the determination of antibodies to HTLV I and II in blood donors, in infected patients and risk individuals, making the determination easier and more reliable.

PRINCIPLE OF THE TEST

The kit uses two highly purified recombinant HTLV Antigens, directed to specific antibodies anti-HTLV, one adsorbed onto the wells of the microplate and the second labeled with peroxidase (HRP). The sample and the Conjugate are added simultaneously to the plate in the 1st incubation. The presence of a specific immunocomplex on the solid phase is detected by the action of the captured Conjugate on the Chromogen/Substrate solution in the 2nd incubation. The intensity of the color generated by the enzyme is proportional to the amount of antibodies in the sample. A cut-off value allows the discrimination between the negative and the positive population.

MATERIALS AND COMPONENTS PROVIDED

• **Strip Microplate** Microplate(s) of 8 x 12 strips of breakable wells coated with Recomb. Antigens HTLV I and II. The microplates are sealed in an aluminum pouch in presence of desiccant bag. no. of microplates: 1
• **Positive Control** – Ready to use. Chimeric buffered solution reactive to anti-HTLV I and II antibodies. It contains 0.09 % sodium azide and 0.09 % Kathon as preservatives and Coomassie brilliant blue as coloring agent. Volume 0.6 ml
• **Negative Control** – Ready to use. Chimeric buffered solution not reactive to anti-HTLV I and II antibodies. It contains 0.09 % sodium azide and 0.09 % Kathon as preservatives. Volume 1.0 ml
• **Washing Solution** – To dilute before use. Solution 25x concentrated that contains Imidazole buffer and surfaceactive agents. Volume 50.0 ml
• **Conjugate** – To dilute before use. Buffered proteic solution, 20x concentrated, that contains polyclonal antibodies antiHAV, labelled with HRP, proteicstabilizers, 0.02% gentamicin sulfate and 0.09 % Kathon as preservatives. Volume 0.4ml
• **Conjugate Diluent** – Buffered proteic solution, for the dilution of the concentrated Conjugate that contains proteic stabilizers, 0.02% gentamicin sulfate and 0.09 % Kathon as preservatives and Ponceau red as coloring agent. Volume 8.0 ml
• **Chromogen** – To mix with Substrate. Solution that contains 3,3',5,5'-tetramethylbenzidine (TMB) with activators and stabilizers diluted in a phosphate/citrate buffer.
Note - Store protected from light. Volume 7.0 ml
• **Substrate** – To mix with Chromogen. Solution that contains stabilized hydrogen peroxide (H2O2) diluted in a phosphate/citrate buffer. Volume 7.0 ml
• **Stop Solution** Solution of 0.3 M sulfuric acid.
Note - Handle with care. Volume 10.0 ml
• **Package insert** – The present document.
Note All the materials of human origin have been controlled and certified by the supplier to be negative for HBsAg, HCV Ab and HIV Ab.

MATERIALS REQUIRED BUT NOT PROVIDED

• Micropipettes of 20, 100, 300 and 1000 µl with disposable tips.
• Vortex mixer and adsorbent papers.
• Distilled water.
• Timer.
• Incubator set at 37 ± 1 °C (dry or moist heat).
• Automatic or manual microplate washer able to aspirate and dispense volumes of 300 - 400 µl.
• Photometric microplate reader linear up to at least 2 OD and supplied with filters of 450 nm and 620 - 630 nm.

STORAGE CONDITIONS

• The kit must be stored at 2 – 8 °C and used before the expire date declared on the external label.
• The pouch containing the microplate has to be brought to room temperature before opening. Take out from the frame only the strips necessary for the test programmed and store the remaining strips in the same pouch in presence of the desiccant bag. Close hermetically the pouch and store again at 2 - 8 °C. If stored properly, strips are stable for 2 months from opening.
• The diluted Washing solution, at room temperature, is stable for 1 week.
• The Chromogen/Substrate are stable until the expiration of the kit.
• The other reagents can be used every time, if stored at 2 – 8 °C and handled carefully for avoiding contamination.

PRECAUTIONS

1. All the reagents contained in the kit are for in vitro diagnostic use only.
2. Do not use the kit or reagents after the expiration date stated on labels.
3. Do not mix reagents of different lots.
4. Procedures should be performed carefully in order to obtain reliable results and clinical interpretations.
5. Bring all the reagents to room temperature for at least 60 minutes, before the test is started.
6. Avoid any contamination of reagents when taking them out of vials. We recommend to use automatic pipettes and disposable tips. When dispensing reagents, do not touch the wall of microplate wells with tips, in order to avoid any crosscontamination.
7. In the washing procedure, use only the Washing Solution provided with the kit and follows carefully the indications reported in the "Washing Instructions" Section of this insert.
8. Ensure that the Chromogen/Substrate does not come in contact with oxidizing agents or metallic surfaces; avoid any intense light exposure during the incubation step or the reagent preparation.
9. Put the reagents in a glass or plastic disposable container, washed with sulfuric acid 1N, then with deionized water, before use.
10. Samples and materials potentially infective have to be handled with care as they could transmit infection. All objects come in direct contact with samples and all residuals of the assay should be treated or wasted as potentially infective. Best procedures for inactivation are treatments with autoclave at 121 °C for 30 minutes or withsodium hypochlorite at a final concentration of 2.5 % for 30minutes. This last method can be used for the treatment of theliquid waste after that it has been neutralized with NaOH.
11. Avoid any contact of liquids with skin and mucousmembrane. Use always protective talk-free gloves, glassesand laboratory coats, according to the safety regulations.
12. Some reagents of the kit contain sodium azide which may betoxic if ingested. Sodium azide may react with copper andlead piping to form highly explosive salts. On disposal, flush with large quantities of water.
13. At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming. Take out from the frame only the strips necessary for the test programmed and store the remaining strips in the same pouch in presence of the desiccant bag.
14. Distribution and incubation times should be the same for all the wells; avoid long interruptions among the different steps of the assay.
15. It is suggested to eliminate the excess of washing solution from wells by blotting them gently on a paper adsorbent pad.
16. The color developed in the last incubation is stable for maximum 1 hour in the dark.
17. We recommend reading the microplate at 450 nm (reading filter) and subtracting the blank at 620 - 630 nm (blanking filter). Blank the reader on A1 well.

SPECIMEN COLLECTION

Either fresh sera or plasma (EDTA, Heparin, Citrate) can be used for the assay. If not used immediately, they can be stored at 2 - 8 °C for 1 week. In case of longer storage freeze them at – 20 °C. Samples should be clear. If the samples are turbid, could be contaminated by micro-organism, insofar it recommends to centrifuge them at 2000 rpm x 20 minutes at room temperature or filtrate on 0.22 µm filters. The samples that, after the above said procedure, did not became clear, cannot be used.

REAGENT PREPARATION

• **Washing Solution** - The concentrated solution to be diluted 1:25 with distilled water.
• **Chromogen/Substrate** - About 5 minutes before use, mix 1 volume of Chromogen with 1 volume of Substrate, in a disposable plastic container, according to needs. This solution is stable for 4 hours at room temperature protected from light.
• **Conjugate** Dilute the concentrated Conjugate 1:20 with the Conjugate Diluent. Mix on vortex before use. The diluted Conjugate is stable for 1 week at 2 – 8 °C, when stored in a sterile disposable container.

WASHING INSTRUCTION

A good washing procedure is essential to get correct and reliable analytical results. In case of manual washing, it is suggested to carry out 5 cycles, first dispensing and then aspirating 300 µl/well per cycle. Usually 5 cycles of automatic washing of 300 µl/well per cycle are sufficient to remove false positives and high background values. It is suggested to use an Elisa automatic microplate washer, qualified and properly serviced. Anyhow, we recommend to calibrate the washing system on the kit itself so to match the declared analytical performances. Any case, potentially infective wastes from microplate washing must be inactivated with Na-hypochlorite at 2.5% final concentration for 30 minutes. All these materials have to be discarded according to the law as potentially infective wastes.

TEST PROCEDURE

At least 1 hour before use bring all the reagents necessary tothe test to room temperature and mix carefully the liquidreagents supplied on vortex (in particular the Controls, theConjugate and the Chromogen/Substrate) avoiding foaming.Do not dilute Controls as they are ready to use.
1. Distribute 50 µl of Controls and Samples not diluted according to the scheme.
2. Add 25 µl of the Conjugate to all the wells, except to blanking well A1
3. Incubate the microplate for 120 minutes at 37 °C sealed with the cardboard sealer.
4. Peel out the plate sealer and wash the microplate according to instructions.
5. Add 100 µl of the Chromogen/Substrate to all the wells, blanking well included
6. Incubate the microplate for 15 minutes at room temperature in the dark.
7. Stop the enzymatic reaction by adding 100 µl Stop Solution to all the wells.
8. Read the microplate at 450 nm and 620 - 630 nm blanking the instrument on blanking well.
Note - Read the microplate within 30 minutes after the dispensing of the Stop Solution

ASSAY SCHEME

At least 1 hour before use bring all the reagents necessary to the test to room temperature and mix carefully the liquid reagents supplied on vortex (in particular the Controls, the Conjugate and the Chromogen/Substrate) avoiding foaming.

HTLV 1+2 Ab
ELISA TEST SYSTEM

Position	Controls/Samples
A1	Blanking well
B1+C1+D1	Negative Control
E1	Positive Control
F1.....H12	Diluted samples

Reagents	Blank	Controls	Samples
Sample	-	-	50 µl
Controls	-	50 µl	-
Conjugate	-	25 µl	25 µl
Cover with the sealer and incubate for 120 minutes at 37 °C			
Peel out the sealer and wash 5 cycles with 300 µl/well per cycle Prepare the necessary Chromogen/Substrate solution.			
Chromogen/Substrate	100 µl	100 µl	100 µl
Incubate for 15 minutes at room temperature in the dark			
Stop Solution	100 µl	100 µl	100 µl
Blank the reader on A1 well. Read at 620 - 630 nm for measuring the microplate background, then at 450 nm.			
The microplate reading must be done within 30 minutes from the Stop Solution dispensing.			

CALCULATION OF RESULTS

If the validity of the assay is confirmed, calculate the Cut-off (Co) value through the following formula:

Cut-off = Negative Control mean + 0.100

Example of calculation

Negative Control mean OD 450 nm 0.050
Positive Control mean OD 450 nm 1.200
Cut-off = NC + 0.100 = 0.150
Sample # 1 OD 450 nm = 0.080 negative
Sample # 2 OD 450 nm = 1.158 positive

Samples with an OD 450 nm value lower than the Co – 15% are classified as negative for anti-HTLV I and II antibodies. Samples with an OD 450 nm value within the Co ± 15% are considered in a grey zone. Samples with an OD 450 nm value higher than the Co + 15% are classified as positive for anti-HTLV I and II antibodies.

Note – The samples in the grey zone must be tested again for confirming them.

VALIDITY OF THE ASSAY

The assay is considered valid if:

1. the OD 450 nm of the A1 blank well is < 0.100. Higher values are index of Chromogen/Substrate contamination;

2. After blanking on A1, the OD 450 nm mean value of the Negative Control (NC) is < 0.100. Abnormal values may be observed when the washing instrument does not work correctly or the washing procedure has not been adapted to the assay as described in the proper section.

3. The OD 450 nm mean value of the Positive Control (PC) is > 0.500. Lower values can be result when the storage temperature was not optimal or with a not correct operative procedure.

In case that the above data do not match the correct values, before repeating the test check carefully the expire date of the kit, the performances of the instruments used for the assay and the procedure of distribution of Controls and samples.

PERFORMANCE CHARACTERISTICS

Sensitivity - The sensitivity of the assay has been calculated on a panel of sero conversion and of positive samples by comparing with a FDA approved kit on the market. The test shows a sensitivity ≥ 98 %.

Specificity - It has been calculated on panels of negative samples, pre-classified with an FDA approved kit present on the market. The assay shows a specificity ≥ 98 % on plasma and sera.

Reproducibility - A set of negative and positive samples is repeatedly tested in different days in order to determine the statistical values of reproducibility for evaluating the interassay variance. The mean value of CV% for OD 450 nm higher than 1.000 (Positive Samples) is lower than 10 %, the mean value of CV% for OD 450 nm lower than 0.100 (Negative Samples) is lower than 30 %.

Repeatability – A set of evaluation intra-assay of negative donor specimens, low and high positive specimens, gives a CV% value ≤ 30% for the negative, ≤ 20% for the low positive and ≤ 10% for the high positive.

LIMITATION OF THE PROCEDURE

Highly lipemic, icteric, hemolysed samples or repeatedly defrost samples and therefore subject to contamination, should not be used as they can give false results in the assay.

The test is for research use only.

PROCEDURE AUTOMATION

This procedure can be used with an automatic device under customer's responsibility and providing he validates the results with an adequate method. For more information, please contact the automatic device manufacturer.

PRECAUTIONS IN USE

The use of the laboratory reagents according to Good Laboratory Practice (GLP) is recommended

WASTE MANAGEMENT

Please, refer to local legal requirements.

REFERENCES

1. Wachsman W and al. - Semin Hematol 1986, 23 - 246 (1956).
2. Ehrlich G. D. and al. - Clin Lab Med 8, 65- 84 (1988)
3. Kalyanarman V. S. et al.. Science 218, 571 - 573 (1982)
4. Lee H. and al. - Science 244, 471 - 475, (1989)
5. Williams A. E. and al. - Science 240, 643 - 646 (1988)
6. Anderson D. W. and al. - Blood 74, 2585 - 91 (1989)
7. Lerner R. A. and al. – Adv. Immunol. 36, 1- 44 (1984)
8. Norrby E. and al.- Nature 329, 248 - 250 (1987)
9. Lee T. and al. – Proc. Nat'l. Acad. Sci. USA 81, 3856 – 60 (1984)

PRESENTACIÓN:

CONT. 96 TEST CODIGO: RSET065-2