DRG® HTLV I&II Ab (EIA-3905)

EU: CE 0318

Revised 21 July 2009 (Vers. 3.0) **RUO in the USA**

Please use only the valid version of the package insert provided with the kit.

INTENDED USE

Third generation Enzyme Immuno Assay (ELISA) for the determination of antibodies to Human T-cell Lymphotropic Virus type I&II or HTLV I&II Ab. The kit may be used for the screening of blood units and the follow-up of HTLV I&II-infected patients.
For "in vitro" diagnostic use only. In the United States, this kit is intended for Research Use Only.

INTRODUCTION

HTLV I&II are retroviruses not related genetically to HIV1&2; however, they have similar routes of transmission and can have extremely long period of latency prior to manifestation of disease.
HTLV I is endemic in southern Japan, the Caribbean and the US and many other scattered population trough the world. HTLV II is endemic in some native American populations but is detected mostly in intravenous drug users and their sexual partners. HTLV I&II are transmitted transplacentally, parenterally, by sexual contacts and by infected blood.
ELISA has been applied to the diagnosis of HTLV I&II serology by detecting specific antibodies in plasma and sera.

PRINCIPLE OF THE TEST

Microplates are coated with HTLV I&II-specific peptides (gp46-I, gp46-II and p21-I).
The solid phase is first treated with the diluted sample and anti HTLV I&II Ab are captured, if present, by the antigens coated on the microplate.
After washing out all the other components of the sample, in the 2nd incubation bound anti HTLV I&II antibodies, IgG and IgM as well, are detected by the addition of polyclonal specific anti hIgG&M antibodies, labelled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti HTLV I&II antibodies present in the sample.
A cut-off value let optical densities be interpreted into anti HTLV I&II antibody negative and positive results.

COMPONENTS

HTLV I&II Ab Elisa contains reagents for 96 tests.

1. Microplate MICROPLATE
n° 1 microplates. 12 strips of 8 breakable wells coated with HTLV I&II specific peptides. Plates are sealed into a bag with desiccant.

2. Negative Control CONTROL –
1 x 2.0 ml/vial. Ready to use control.
It contains goat serum proteins, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. The negative control is olive green color coded.

3. Positive Control CONTROL +
1 x 2.0 ml/vial. Ready to use control.
It contains 1% goat serum proteins, human antibodies positive to HTLV I&II, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. The Positive Control is dark green color coded.

4. Calibrator CAL ….
n° 1 vials. Lyophilized calibrator.
To be dissolved with the volume of EIA grade water reported on the label.
It contains human antibodies to HTLV I&II whose content is calibrated on Accurun 1 series 4100 (Boston Biomedica Inc., USA, lot # 104978), 50 mM Tris buffer pH 7.8, 4% Bovine serum albumin, 2% Mannitol, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

5. Wash buffer concentrate WASHBUF 20X
1 x 60 ml/bottle. 20x concentrated solution
containing 0.1% Kathon GC as preservative.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2 and 0.05% Tween 20 .

6. Enzyme Conjugate CONJ
1 x 16 ml/vial. Ready to use and red colour coded reagent.
It contains Horseradish Peroxidase conjugated goat polyclonal antibodies to human IgG and IgM, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

7. Chromogen/Substrate SUBS TMB
1 x 16 ml/vial. Ready-to-use component.
It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide or H_2O_2.
Note: To be stored protected from light as sensitive to strong illumination.

8. Assay Diluent DILAS
1 x 8 ml/vial.
10 mM tris buffered solution pH 8.0 +/-0.1 containing 0.1% Kathon GC for the pre-treatment of samples and controls in the plate, blocking interference.

9. Sulphuric Acid H_2SO_4 0.3 M
1 x 15 ml/vial. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (Xi R36/38; S2/26/30).

10. Sample Diluent DILSPE
1 x 50 ml/vial.
It contains 1% goat serum proteins, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives.
To be used to dilute the sample.
Note: The diluent changes colour from olive green to dark bluish green in the presence of sample.

11. Plate sealing foils n° 2

12. Package insert n° 1

MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (200 µl and 10µl) and disposable plastic tips.
2. EIA grade water (bidistilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator capable to provide a temperature of +37°C.
6. Calibrated ELISA microwell reader with 450 nm (reading) and possibly with 620-630 nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. When the kit is used for the screening of blood units and blood components, it has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
3. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
4. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
5. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate from strong light and avoid vibration of the bench surface where the test is undertaken.
6. Upon receipt, store the kit at 2 - 8°C into a temperature controlled refrigerator or cold room.
7. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
8. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
9. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
10. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
11. Do not use the kit after the expiration date stated on the external container and internal (vials) labels.
12. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
13. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.

14. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
15. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
16. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water.
17. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

SPECIMEN: PREPARATION AND RECOMMENDATIONS

1. Blood is drawn aseptically by venipuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
4. Hemolyzed (red) and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°C - 8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8µ filters to clean up the sample for testing.

PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-use of the device and up to 6 months.

1. Microplates:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of storage. In this case call the customer service. Unused strips have to be placed back into the aluminum pouch, in presence of desiccant supplied, firmly zipped and stored at +2°C - 8°C. When opened the first time, residual strips are stable till the indicator of humidity inside the desiccant bag turns from yellow to green.

2. Negative Control:

Ready to use. Mix well on vortex before use.

3. Positive Control:
Ready to use. Mix well on vortex before use. Handle this component as potentially infective, even if HTLV I&II, if present in the control, has been chemically inactivated.

4. Calibrator:
Dissolve carefully the content of the lyophilized vial with the volume of EIA grade water reported on its label. Mix well on vortex before use.
Handle this component as potentially infective, even if HTLV I&II, if present in the control, has been chemically inactivated.
Note: *When dissolved the Calibrator is not stable. Store in aliquots at –20°C.*

5. Wash buffer concentrate:
The 20x concentrated solution has to be diluted with EIA grade water up to 1200 ml and mixed gently end-over-end before use. As some salt crystals may be present into the vial, take care to dissolve all the content when preparing the solution.
In the preparation avoid foaming as the presence of bubbles could give origin to a bad washing efficiency.
Note: *Once diluted, the wash solution is stable for 1 week at +2°C - 8°C.*

6. Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly sterile disposable containers.

7. Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container.

8. Assay Diluent:
Ready to use. Mix well on vortex before use.

9. Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (Xi R36/38; S2/26/30)
Legenda: R 36/38 = Irritating to eyes and skin.
S 2/26/30 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

10. Sample Diluent:
Ready to use. Mix well on vortex before use.

INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the sections "Validation of Test" and "Assay Performances". Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of ±5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and ideally with a second filter (620-630nm) for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; repeatability ≥ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended for blood screening when the number of samples to be tested exceed 20-30 units per run.

7. When using automatic devices, in case the vial holder of the instrument does not fit with the vials supplied in the kit, transfer the solution into appropriate containers and label them with the same label peeled out from the original vial. This operation is important in order to avoid mismatching contents of vials, when transferring them. When the test is over, return the secondary labeled containers to 2°C – 8°C, firmly capped.
8. DRG's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
7. Check that the ELISA reader has been turned on at least 20 minutes before reading.
8. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

Automated assay:
In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 200 µL Sample Diluent and then 10 µL sample.
All the mixture is then carefully dispensed directly into the appropriate sample well of the microplate. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples.
Do not dilute controls/calibrator as they are ready to use.
Dispense 200 µL controls/calibrator in the appropriate control/calibration wells.
Important Note: *Visually monitor that samples have been diluted and dispensed into appropriate wells. This is simply achieved by checking that the color of dispensed samples has turned to dark bluish-green while the color of the negative control has remained olive green.*
For the next operations follow the operative instructions reported below for the Manual Assay.
It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

Manual assay:

1. Place the required number of Microwells in the microwell holder. Leave the 1st well empty for the operation of blanking.
2. Dispense 200 µL of Negative Control in triplicate,
   200 µL Calibrator in duplicate and
   200 µL Positive Control in single in proper wells.
   Do not dilute Controls and Calibrator as they are pre-diluted, ready to use !
3. Add 200 µL of Sample Diluent (DILSPE) to all the sample wells;
   then dispense 10 µL sample in each properly identified well.
   Mix gently the plate, avoiding overflowing and contaminating adjacent wells, in order to fully disperse the sample into its diluent.
   Important note: *Check that the color of the Sample Diluent, upon addition of the sample, changes from light green to dark bluish green, monitoring that the sample has been really added.*
4. Dispense 50 µL Assay Diluent (DILAS) into all the controls/calibrator and sample wells. Check that the color of samples has turned to dark blue.
5. Incubate the microplate for **45 min at +37°C**.
   Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*
6. Wash the microplate with an automatic washer by delivering and aspirating 350 µl/well of diluted washing solution as reported previously (section 9.3).
7. Pipette 100µl Enzyme Conjugate into each well, except the 1st blanking well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.
   Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*
8. Incubate the microplate for **45 min at +37°C**.
9. Wash microwells as in step 6.
10. Pipette 100 µL Chromogen/Substrate mixture into each well, the blank well included.
    Then incubate the microplate at **room temperature (18-24°C) for 15 minutes**.
    Important note: *Do not expose to strong direct illumination. High background might be generated.*
11. Pipette 100µL Sulphuric Acid into all the wells using the same pipetting sequence as in step 10 to stop the enzymatic reaction. Addition of acid will turn the positive control and positive samples from blue to yellow.
12. Measure the color intensity of the solution in each well, as described in section 9.5, at 450nm filter (reading) and possibly at 620-630nm (background subtraction), blanking the instrument on A1.

Important notes:

1. *If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*
3. *Shaking at 350 ± 150 rpm during incubation has been proved to increase the sensitivity of the assay of about 20%.*

ASSAY SCHEME

Method	Operations
Controls & Calibrator Samples Assay Diluent (DILAS)	200 µL 200 µL dil.+10 µL 50 µL
1st incubation	**45 min**
Temperature	+37°C
Wash step	4-5 cycles
Enzyme conjugate	100 µL
2nd incubation	**45 min**
Temperature	+37°C
Wash step	4-5 cycles
TMB/H2O2	100 µL
3rd incubation	**15 min**
Temperature	r.t.
Sulphuric Acid	100 µL
Reading OD	450nm

An example of dispensation scheme is reported below:
Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL	S6										
F	CAL	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank NC = Negative Control CAL = Calibrator POS = Positive Control S = Sample

INTERNAL QUALITY CONTROL

A check is carried out on the controls and the calibrator any time the kit is used in order to verify whether their OD450nm values are as expected and reported in the table below.

Check	Requirements
Blank well	< 0.100 OD450nm value
Negative Control (NC)	< 0.050 mean OD450nm value after blanking
Calibrator	S/Co > 1.1
Positive Control	> 1.000 OD450nm value

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
Negative Control (NC) > 0.050 OD450nm after blanking	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control; 4. that no contamination of the negative control or of their wells has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator S/Co < 1.1	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of negative control instead 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.

Positive Control < 1.000 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in the distribution of controls (dispensation of negative control instead of positive control. In this case, the negative control will have an OD450nm value > 0.150, too. 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

Should these problems happen, after checking, report any residual problem to the supervisor for further actions.

CALCULATION OF THE CUT-OFF

The tests results are calculated by means of a cut-off value determined with the following formula on the mean OD450nm value of the Negative Control (NC):

NC + 0.350 = Cut-Off (Co)

The value found for the test is used for the interpretation of results as described in the next paragraph.
Important note: *When the calculation of results is done by the operative system of an ELISA automated work station be sure that the proper formulation is used to calculate the cut-off value and generate the right interpretations of results.*

INTERPRETATION OF RESULTS

Test results are interpreted as ratio of the sample OD450nm and the Cut-Off value (or S/Co) according to the following table:

S/Co	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient has not been infected by HTLV I&II or that the blood unit may be transfused.
Any patient showing an equivocal result should be tested again on a second sample taken 1-2 weeks later from the patient and examined. The blood unit should not be transfused.
A positive result is indicative of HTLV I&II infection and therefore the patient should be treated accordingly or the blood unit should be discarded.

Important notes:

1. *Interpretation of results should be done under the supervision of the responsible of the laboratory to reduce the risk of judgment errors and misinterpretations.*

2. *Any positive result should be confirmed by an alternative method capable to detect IgG and IgM antibodies (confirmation test) before a diagnosis of HTLV infection is formulated.*
3. *When test results are transmitted from the laboratory to an informatics centre, attention has to be done to avoid erroneous data transfer.*
4. *Diagnosis of HTLV I&II infection has to be done and released to the patient only by a qualified medical doctor.*

An example of calculation is reported below:

The following data must not be used instead or real figures obtained by the user.

Negative Control: 0.019 – 0.020 – 0.021 OD450nm
Mean Value: 0.020 OD450nm
Lower than 0.050 – Accepted

Positive Control: 2.189 OD450nm
Higher than 1.000 – Accepted
Cut-Off = 0.020+0.350 = 0.370

Calibrator: 0.830 - 0.870 OD450nm
Mean value: 0.850 OD450nm S/Co = 2.3
S/Co higher than 1.1 – Accepted

Sample 1: 0.070 OD450nm
Sample 2: 1.690 OD450nm
Sample 1 S/Co < 0.9 = negative
Sample 2 S/Co > 1.1 = positive

PERFORMANCES

Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC).

LIMIT OF DETECTION

The limit of detection of the assay has been calculated by means of the preparation Accurun 1 series 4100, lot # 104978, produced by Boston Biomedica Inc., USA.
The table below reports the mean OD450nm values of this material when used undiluted.

Lot #	**1203**	**Lot #**	**0604**	**Lot #**	**0904**
OD450nm	S/Co	OD450nm	S/Co	OD450nm	S/Co
0.458	**1.3**	0.534	**1.5**	0.482	**1.4**

DIAGNOSTIC SPECIFICITY AND SENSITIVITY

The Performance Evaluation of the device was carried out in a trial conducted on more than total 5000 samples.

Diagnostic specificity

It is defined as the probability of the assay of scoring negative in the absence of specific analyte. A total of more 5000 unselected donors, including 1st time donors, were examined.
The diagnostic specificity was assessed against a kit US FDA approved.
About 5000 blood donors were tested providing a specificity equal or better than 99.5%.
210 hospitalized patients were tested for HTLV Ab; a diagnostic specificity of at least 99.5% was found.

Moreover, diagnostic specificity was assessed by testing 162 potentially interfering specimens (other infectious diseases, E.coli antibody positive, patients affected by non viral hepatic diseases, dialysis patients, pregnant women, hemolized, lipemic, etc.).
A value of specificity of at least 99.5% was assessed.

No false reactivity due to the method of specimen preparation has been observed.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the value of specificity.
Frozen specimens have been tested, as well, to check for interferences due to collection and storage.
No interference was observed.

Diagnostic Sensitivity

It is defined as the probability of the assay of scoring positive in the presence of specific analyte.
The diagnostic sensitivity has been assessed in the Performance Evaluation on a total number of more than 400 specimens coming from both HTLV I and HTLV II infection; a diagnostic sensitivity of 100% was found.

Furthermore, the performance panel code PRP 206 available from Boston Biomedica Inc., was studied.
Results are reported in the following table.

BBI – Panel code PRP 206

Member N°	Result	Lot # 1203 S/Co	Lot # 0604 S/Co	Lot # 0904 S/Co	Rif. BMX S/Co
1	+	7.6	8.3	8.5	4.2
2	+	6.7	7.5	7.0	5.2
3	+	11.3	11.3	11.2	7.0
4	+	5.6	7.3	7.0	5.1
5	+	2.8	3.3	3.0	4.1
6	+	5.8	7.2	6.7	4.5
7	+	5.8	7.0	8.0	4.3
8	+	11.3	11.3	11.2	7.1
9	+	2.2	2.2	3.0	5.2
10	-	0.2	0.2	0.2	0.3
11	+	1.1	1.4	1.2	3.3
12	+	11.3	11.3	11.2	2.4
13	+	11.3	11.3	11.2	7.2
14	+	2.7	2.8	3.2	2.9
15	+	11.3	11.3	11.2	6.2

PRECISION

It has been calculated on two samples, one negative and one low positive, examined in 16 replicates in three separate runs. Results are reported as follows:

Lot # 1203

Negative Sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.062	0.071	0.068	0.067
Std.Deviation	0.010	0.001	0.005	0.005
CV %	15.9	2.0	7.3	8.4

Calibrator (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.857	0.929	0.881	0.889
Std.Deviation	0.007	0.020	0.029	0.019
CV %	0.8	2.1	3.3	2.1
S/Co	2.4	2.6	2.	2.5

Lot # 0604

Negative Sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average
OD 450nm	0.058	0.077	0.061	0.066
Std.Deviation	0.011	0.008	0.007	0.009
CV %	19.5	10.1	11.5	13.7

Calibrator (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.858	0.834	0.899	0.864
Std.Deviation	0.004	0.006	0.010	0.006
CV %	0.41	0.68	1.10	0.73
S/Co	2.4	2.4	2.6	2.5

Lot # 0904

Negative Sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average
OD 450nm	0.057	0.073	0.072	0.067
Std.Deviation	0.008	0.002	0.001	0.004
CV %	14.99	2.90	1.97	6.62

Calibrator (N = 16)

Mean values	1st run	2nd run	3rd run	Average
OD 450nm	0.872	0.937	0.911	0.907
Std.Deviation	0.029	0.004	0.021	0.018
CV %	3.3	0.5	2.3	2.0
S/Co	2.48	2.65	2.59	2.57

The variability shown in the tables above did not result in sample misclassification.

LIMITATIONS

Repeatable false positive results, not confirmed by Western Blot or similar confirmation techniques, were assessed as less than 0.1% of the normal population.
Frozen samples containing fibrin particles or aggregates after thawing have been observed to generate some false results.

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DRG® HTLV I&II Ab (EIA-3905)

EU: CE 0318

Revised 21 July 2009 (Vers. 3.0) **RUO in the USA**

Symbols used with DRG Assays

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..