IVD CE

# Sclavo-EIA HTLV I/II Ab Screen

## Ref ELI904001

Instructions for Use

**Rev. D ELI904001-05-2022**

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## A. INTENDED USE

Sclavo-EIA HTLV I/II Ab screen is an ELISA kit for the simultaneous screening of total antibody against Human T Cell Leukemia (HTLV) types 1 and 2 in human plasma and serum.
This kit can be used for the screening of samples from blood donors in blood banks and for the follow-up HTLV 1 and 2 infected patients in diagnostic laboratories.

## B. INTRODUCTION

Human T cell leukemia virus type 1 (HTLV-1) was the first discovered human retrovirus in 1980; it is the etiologic agent of adult T-cell leukemia and HTLV-1-associated myelopathy/tropical spastic paraparesis. Human T-cell leukemia virus type 2 (HTLV-2) was isolated from a patient with hairy cell leukemia for the first time in 1982.
There are three primary modes of HTLV transmission: vertical (e.g., at the time of delivery or breast feeding), parenteral (e.g., transfusion of contaminated blood or blood derivatives, transplantation of infected organs, or sharing of infected syringes among intravenous drug users), and sexual.
HTLV 1 is endemic in some parts of the world (Southwestern Japan, South America, the Caribbean Basin, the Middle East, Australo – Melanesia, the West Indies, Jamaica), as well as equatorial Africa. HTLV-2 is less widespread compared to type 1 and is endemic in in Central and West Africa; in native Amerindian populations in North, Central, and South America; and among cohorts of intravenous drug users in the United States and Europe.
ELISA has been applied to the diagnosis of HTLV I&II serology by detecting specific antibodies in plasma and sera.

## C. PRINCIPLE OF THE TEST

The solid phase (microtiter wells) is sensitized with with HTLV-1 and -2 specific synthetic immunodominant antigens derived from gp46-I, gp46-II and gp21.
Samples are added into the microtiter wells and, if antibodies specific to HTLV-1 or HTLV-2 (IgG, IgM or IgA) are present in the sample, they will form stable complexes with the recombinant antigens coated to the solid phase.
After a washing step to remove the unbound proteins, the antibody-antigen complexes are detected by the addition of specific synthetic antigens derived from gp46-I, gp46-II and gp21, labelled with peroxidase (HRP).
The presence of anti-HTLV-1 and anti-HTLV-2 antibodies is revealed by adding the colorless substrate TMB to start the colorimetric reaction. The blue color that is developed is proportional to the quantity of antibody/antigen bound to the solid phase. The enzymatic reaction is stopped with an acidic solution and the color of the reaction mixture turns from blue to yellow.

## D. COMPONENTS OF THE KIT AND THEIR PREPARATION

The kit is for 96 tests and contains the following materials.

**Microtiter plate** **MICROPLATE**

12 strips x 8 wells coated with HTLV-1 and -2 specific synthetic immunodominant antigens derived from gp46-I, gp46-II and gp21 in a resealable pouch with desiccant bag.
Equilibrate the microplate to room temperature (1 hour) before opening the pouch. If the humidity indicator in the desiccant bag has turned dark green, do not use the microplate.
Put the unused strips back into the resealable pouch with the desiccant bag, squeeze to remove the air, firmly close the pouch and store at 2 – 8°C.
After the first opening, strips can be used until the humidity indicator in the desiccant bag has turned yellow to green.

**Negative control** **CONTROL –**

**GHS07**

1 vial x 2 ml. Ready to use. Color code: yellow-brown. Vortex thoroughly before use.
10 mM Phosphate buffer pH 7,4 containing BSA 5%. Preservatives: sodium azide (0,09%) and Proclin™ 300 (0,045%).
**Warning:** ATTENTION - Sensitizing (H317; P261; P280; P362+P364; P333+P313; P321; P501).
*Contains reaction mass of 5-chloro-2-methyl-4-iso-thiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1) (CAS 55965-84-9)*

**Positive control** **CONTROL +**

**GHS07**

1 vial x 2 ml. Ready to use. Color code: light green. Vortex thoroughly before use.
10 mM Phosphate buffer pH 7,4 containing inactivated human serum positive to HTLV Ab, BSA 5%. Preservatives: sodium azide (0,09%) and Proclin™ 300 (0,045%).

**Warning**: ATTENTION - Sensitizing (H317; P261; P280; P362+P364; P333+P313; P321; P501).
*Contains reaction mass of 5-chloro-2-methyl-4-iso-thiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1) (CAS 55965-84-9)*

**Calibrator** **CAL**

**GHS07**

1 vial. Freeze-dried. To be reconstituted with the volume of EIA grade water reported in the label. Vortex thoroughly before use.
Inactivated anti HTLV I/II antibodies, calibrated against Seracare Accurun 24, 4% BSA, 2% Mannitol, 50mM Tris buffer pH 7.8. Preservatives: gentamicin sulphate (0,02%) and Proclin™ 300 (0,045%).
**Important**: *once reconstituted the calibrator is not stable. Make aliquots and store at -20°C up to 6 months.*
**Warning**: ATTENTION – Sensitizing (H317; P261; P280; P362+P364; P333+P313; P321; P501).
*Contains reaction mass of 5-chloro-2-methyl-4-iso-thiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1) (CAS 55965-84-9)*

**Concentrated washing buffer** **WASHBUF 20X**

**GHS09**

**GHS07**

1 bottle x 60 ml. Concentrated 20 times. Bring to the final volume of 1200 ml using EIA grade water.
Once diluted it contains 10 mM phosphate buffer pH 7.0, 0,5% Tween 20 and Proclin™ 300 (0.045 %).
Carefully check that there are no undissolved salt crystals, if necessary, mix until completely dissolved.
**Important**: *avoid the formation of foam during resuspension as it could give rise to false results.*
**Important**: *once reconstituted the ready to use buffer is stable 1 week at 2-8°C.*
**Warning**: DANGER – Sensitizing / Dangerous for the environment ((H317; H411; P101; P102; P103; P261; P273; P280; P333+P313; P321; P501).
*Contains reaction mass of 5-chloro-2-methyl-4-iso-thiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1) (CAS 55965-84-9)*

**Conjugate** **CONJ**

**GHS07**

1 Vial x 16 ml. Ready to use. Color code: pink/red. Mix gently before use.
10 mM Tris buffer pH 6.8, HTLV synthetic antigens mixture, labelled with HRP, BSA 5% Preservatives: gentamicin sulphate (0,02%) and Proclin™ 300 (0,045%).
**Warning**: ATTENTION – Sensitizing (H317; P261; P280; P362+P364; P333+P313; P321; P501).
*Contains reaction mass of 5-chloro-2-methyl-4-iso-thiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1) (CAS 55965-84-9)*

**Substrate** **SUBS TMB**

1 Vial x 16 ml. Ready to use. Vortex thoroughly before use.
0,03% tetramethylbenzidine (TMB), 4% dimethylsulphoxide, and 0,02% hydrogen peroxide ($H_2O_2$) stabilised in 50 mM citrate buffer (pH 3.8).
**Important**: *do not expose to strong illumination, oxidizing agents (e. g. hypochlorite fumes), metallic surfaces; store protected from light.*

**Sulphuric acid** **$H_2SO_4$ 0.3 M**

**GHS05**

1 Vial x 15 ml. Ready to use. Mix by inversion before use.
0.3 M $H_2SO_4$ solution.
**Warning**: DANGER - Corrosive (H314; P303 + P361 + P353; P305 + P351 + P338; P310; P321; P405; P501).
*Contains Sulphuric Acid (CAS 7664-93-9)*

**Legend:**
Warning statements H:
H314 - Causes severe skin burns and eye damage.
H317 - May cause an allergic skin reaction.
H411 - Toxic to aquatic life with long lasting effects.

Precautionary statements P:
P101 - In the event of a doctor's consultation, keep the product container or label available.
P102 - Keep out of reach of children.
P103 – Read carefully and follow all instructions.
P261 - Avoid breathing dust / fume / gas / mist / vapors / spray.
P273 - Do not release into the environment.
P280 - Wear protective gloves.
P310 - Immediately call a Poison Center / doctor.
P321 - Specific treatment (see on this label)
P303 + P361 + P353 - IF ON SKIN (or hair): take off immediately all contaminated clothing. Rinse the skin / take a shower.
P305 + P351 + P338 - IF IN EYES: rinse thoroughly for several minutes. Remove any contact lenses if easy to do. Continue rinsing.
P333 + P313 - If skin irritation or rash occurs: Get medical attention.
P362 + P364 - Take off contaminated clothing and wash it before wearing again.
P405 - Store locked up.
P501 - Dispose of contents / container in accordance with local / regional / national / international regulations.

| | |
|---|---|
| **Adhesive films to cover microplate** | **2** |
| **Instructions for use booklet** | **1** |

**E. Materials Required But Not Provided**

Laboratory glassware: appropriately sized graduated cylinders, pipettes etc.

Adjustable single-channel micropipettes capable of delivering 10 µL and 200 µL and disposable plastic tips.

EIA-grade distilled water.

Dual wavelength microwell reader capable of reading at 450 nm with a reference filter of 620 - 630 nm. If the reference filter is not available, make sure that the bottom of the microtiter wells is clean (do not touch without gloves).

37°C ±1°C incubator (dry or humidified).

Calibrated multichannel ELISA washer device.

Vortex or similar mixing device.

Timer.

Absorbent paper to blot the microtiter plate.

**F. PRECAUTIONS AND WARNINGS**

CAUTION: Some components of this kit contain materials of human origin (blood derivatives) therefore, should be handled as potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory practices.

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. After opening, the stability of the individual reagents is described in section D.
8. 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.
9. 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.

10. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable ti
11. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
12. Do not use the unopened kit after the expiration date indicated on the outer container and inner labels (vials).
13. 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.
14. 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.
15. 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 minutes.
16. 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.
17. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water.
18. 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.

**G. SPECIMEN: TYPE, PREPARATION AND RECOMMANDATIONS.**

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. The use of anticoagulants such as citrate, EDTA and heparin does not interfere with the test.
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 labelling and electronic reading is strongly recommended.
4. Haemolysed (reddish) and visibly strongly lipemic ("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°- 8°C in primary collection tubes for up to five days after collection.
   Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C according laboratory validated sample storage procedures. 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 minutes or filter using 0,2 – 0,8-micron filters to clean up the sample for testing.

**H. 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 in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution. The instrument has to be submitted weekly to decontamination according to its manual (NaOH 0,1 M decontamination suggested). Five (5) washing cycles (aspiration + dispensation of 350 µl/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing. An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of +/- 5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory 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; (d) 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 section O "Internal Quality Control". 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 labelled containers to 2° - 8°C, firmly capped.

**I. 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 reagents beyond their stated expiry date.
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 aluminium 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 freeze-dried 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 manufacturer's instructions. Set the right number of washing cycles as reported in the specific section.
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.

**J. TEST PROCEDURE**

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

**Automated assay:**

Follow the automated ELISA processor user manual for programming the Sclavo-EIA HTLV I/II Ab test method.
In case the test is carried out automatically with an ELISA processor, we recommend to dispense the sample 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.
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 8-Microwell strips in the microwell frame. holder and wash them once to hydrate wells. Carefully identify the wells for controls, calibrator and samples. Leave the 1st well (A1) empty for the blank substrate.
2. Dispense 100 µl Negative Control in triplicate,100 µl Calibrator in duplicate, 100 µl Positive control in single followed by 100 µl for each sample in single in proper wells.
3. Incubate the microplate, sealed with the supplied adhesive foil, for 45 minutes at 37°C.
4. Wash the microplate with an automatic washer by delivering and aspirating 350 µl/well of diluted washing solution as reported previously (section H.3).
5. Pipette 100 µl Conjugate n. 1 into each well, except well A1 (blank substrate), and cover with the adhesive foil.

**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.

6. Incubate the microplate, sealed with the supplied adhesive foil, for 45 minutes at 37°C.
7. Wash the microplate as in step 5.
8. Pipette 100 µl Chromogen/Substrate mixture into each well, blank well A1 included. Then incubate the microplate at room temperature (18-24°C) for 15 minutes.

Start the countdown from the moment the reagent is dispensed into the first reaction well.
**Important note**: Do not expose to strong direct illumination: high background might be generated.

9. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 8 to stop the enzymatic reaction. Addition of acid will turn the positive control and positive samples from blue to yellow/brown.
10. Measure the colour intensity of the solution in each well, as described in section H.5, at 450nm filter (reading) and at 620-630nm (background subtraction), blanking the instrument on A1 (mandatory).

**Important notes:**

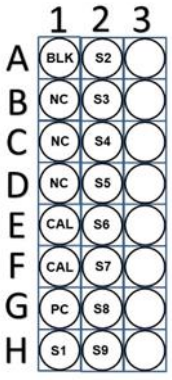
1. Ensure that no fingerprints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.
2. Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self-oxidation of the chromogen can occur leading to high background.
3. The Calibrator (CAL) does not affect the cut-off calculation and therefore the test results calculation. The Calibrator may be used only when a laboratory internal quality control is required by the management.
4. Shaking at 350 ± 150 rpm during incubation has been proved to increase the sensitivity of the assay of about 20%.

## K. ASSAY SCHEME

| Operations | Procedure |
|---|---|
| Controls, Calibrator, Samples | 100 µl |
| **1st incubation** | **45 min** |
| Temperature | +37°C |
| Washing steps | n° 5 cycles with 20'' of soaking<br>OR<br>n° 6 cycles without soaking |
| Conjugate | 100 µl in all wells except A1 |
| **2nd incubation** | **45 min** |
| Temperature | +37°C |
| Washing steps | n° 5 cycles with 20'' of soaking<br>OR<br>n° 6 cycles without soaking |
| TMB/$H_2O_2$ | 100 µl in all wells |
| **3rd incubation** | **15 min** |
| Temperature | Room (18-24°C) |
| Sulphuric acid | 100 µl in all wells |
| Reading OD | 450nm / 620-630nm |

An example of dispensation scheme is reported below:

**Microplate**




**Legenda:**
BLK = Blank
NC = Negative Control
CAL = Calibrator
PC = Positive Control
S = sample

## L. 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.

| Parameter | Requirements |
|---|---|
| Blank well | < 0,100 OD 450 nm value |
| Negative Control (NC) | < 0,150 mean OD 450 nm value<br>after blank subtraction. |
| Calibrator | Index S/CO > 1,5 |
| Positive control | >1,000 OD 450 nm |

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**<br>> 0.100 OD450nm | 1. that the Chromogen/Substrate solution has not become contaminated during the assay. |
| **Negative Control**<br>(NC)<br>> 0,150 OD 450 nm after blanking | 1. that the washing procedure and the washer settings are as validated in the pre-qualification study;<br>2. that the proper washing solution has been used and the washer has been primed with it before use;<br>3. that no mistake has been done in the assay procedure (dispensation of positive control instead of the negative one);<br>4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to spills of positive samples or of the enzyme conjugate;<br>5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate;<br>6. that the washer needles are not blocked or partially obstructed. |
| **Calibrator**<br>S/CO < 1,500 | 1. that the procedure has been correctly performed;<br>2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead of positive control);<br>3. that the washing procedure and the washer settings are as validated in thep re qualification study;<br>4. that no external contamination of the calibrator has occurred. |
| **Positive control**<br>< 1,000 OD 450 nm | 1. that the procedure has been correctly performed;<br>2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of the calibrator. In this case, the negative control will have an OD450nm value > 0.200 too);<br>3. that the washing procedure and the washer settings are as validated in the pre-qualification study;<br>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.

**M. 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/620 – 630nm value of the Negative Control (NC):

**NC + 0,200 = 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.

## N. 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 anti HTLV antibodies (RIBA or similar), and possibly with a Molecular Biology assay, before a diagnosis of HTLV infection is formulated.
3. When test results are transmitted from the laboratory to another department, attention must be paid to avoid erroneous data transfer.
4. Diagnosis of HTLV infection has to be taken and released to the patient by a suitably 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** | OD 450 nm: 0,079 – 0,080 – 0,081 |
| OD 450 nm mean value: 0,080 | Lower than 0,150 |
| ACCEPTED | |
| **CUT-OFF** | 0,200 + 0,080 = 0,280 |
| **POSITIVE CONTROL** | OD 450 nm: 2,479-2,481 |
| OD 450 nm mean value: 2,480 | Higher than 1,000 |
| ACCEPTED | |
| **CALIBRATOR** | OD 450 nm: 1,105 – 1,115 |
| OD 450 nm mean value: 1,110 S/CO = 3,96 | Higher than 1,5 |
| ACCEPTED | |
| Sample 1 OD 450 nm: 0,048 S/CO = 0,17 | S/CO < 0,9 |
| NEGATIVE | |
| Sample 2 OD 450 nm: 1,48 S/CO = 5,29 | S/CO > 1,1 |
| POSITIVE | |

## O. 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).

**O.1. Analytical sensitivity**

The limit of detection of the assay has been calculated by means of the preparation Accurun 24, lot # 118956, produced by Seracare Life Sciences, USA.
The table below reports the results obtained for this material with three lots of products (P1, P2 and P3)
Accurun 24 was diluted in HTLV Ab negative serum and examined in 4 replicates.

| Sample dilution | Batch P1 | Batch P2 | Batch P3 |
|---|---|---|---|
| | OD 450 nm | | |
| 1:4 | **2,981** | **2,957** | **3,455** |
| 1:8 | **1,964** | **1,856** | **1,992** |
| 1:16 | **0,935** | **0,820** | **0,971** |
| 1:32 | **0,551** | **0,453** | **0,562** |
| 1:64 | **0,318** | **0,357** | **0,434** |
| 1:128 | 0,201 | 0,195 | 0,251 |
| Diluent | 0,040 | 0,059 | 0,055 |

**O.2. Diagnostic specificity and sensitivity.**

The Performance Evaluation of the device was carried out in a trial conducted on more than total 5000 samples, according to the requirements of the CTS:2009.
Further tests were carried out internally on commercially available panels of characterized positive samples.

**O.3 Diagnostic Specificity:**

It is defined as the probability of the assay of scoring negative in the absence of specific analyte. In the first part of the study, a total of more 5000 samples, including unselected donors, hospitalized patients and potentially cross-reacting specimens, were examined, matching the requirements of CTS:2009, the resulting specificity was 100%.Tthe diagnostic specificity was recently assessed by testing a total of 3354 negative samples on five different lots. A value of specificity of 100% was observed.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera were tested as well to assure no interference due to the sample preparation. Frozen specimens have been tested, as well, to check for interferences due to collection and storage.
No false reactivity due to the method of specimen preparation has been observed.

**O.4 Diagnostic Sensitivity**

It is defined as the probability of the assay of scoring positive in the presence of specific analyte.
The diagnostic sensitivity was assessed in the internal Performance Evaluation on a total number of more than 400 specimens coming from both HTLV I (n=305) and HTLV II (n=112) infected patients.
The diagnostic sensitivity was additionally evaluated on
- The panel code PRP 207/M supplied by BBI, USA;
- Two panels produced by EFS, France, and based on samples of European origin;
- NIBSC UK – Monitor Sample for anti-HTLV-I, Lot 03/104-009.

Using the HTLV I/II ELISA kit manufactured by Murex (values shown as reported in the leaflet of the panel or tested internally using Murex kit); Samples were evaluated in duplicate (n = 2).

A diagnostic sensitivity of 100% was found.

Results obtained on panels are reported as follows:

**BBI Panel code PRP 207/M**

| Sample N° | Result | KIT S/CO | Murex S/CO |
|---|---|---|---|
| 1 | **POS** | **15.1** | **11.8** |
| 2 | **POS** | **8.0** | **11.6** |
| 3 | **POS** | **13.3** | **11.6** |

| | | | |
|---|---|---|---|
| 4 | **POS** | **17.4** | **11.8** |
| 5 | **POS** | **17.4** | **11.6** |
| 6 | / | / | / |
| 7 | **POS** | **17.4** | **11.6** |
| 8 | **POS** | **17.4** | **11.6** |
| 9 | **POS** | **17.4** | **11.6** |
| 10 | **POS** | **17.0** | **11.6** |
| 11 | **POS** | **10.4** | **11.8** |
| 12 | **POS** | **17.4** | **11.6** |
| 13 | **POS** | **17.4** | **11.6** |
| 14 | **POS** | **17.4** | **11.6** |
| 15 | NEG | 0.5 | 0.2 |

**EFS – Panel Ac HTLV lot # 07.140625**

| Member N° | Kit S/CO | Murex S/CO |
|---|---|---|
| 1 | **1.5** | **4.2** |
| 2 | **7.6** | **6.9** |
| 3 | **4.7** | **8.6** |
| 4 | **4.0** | **8.1** |
| 5 | **2.5** | **4.5** |
| 6 (diluent) | 0.2 | 0.2 |

**EFS – Panel Ac HTLV lot # 05/08.2012.22C**

| Member N° | Kit S/CO | Murex S/CO | typing |
|---|---|---|---|
| 1 | **3.4** | **10.1** | p19I/p24/gp46I |
| 2 | **12.2** | **13.3** | p19I/gp46I/gp21 |
| 3 | **10.6** | **9.4** | gp46I |
| 4 | **11.1** | **9.6** | gp46II |

**NIBSC – Lot 03/104-009**

| P1 | P2 | P3 | Murex |
|---|---|---|---|
| S/CO | S/CO | S/CO | S/CO |
| **1.8** | **1.3** | **1.9** | **2.1** |

**O.5 Precision**

The Negative Control (NC), the Calibrator (CAL) and the Positive Control (PC) of the device were examined in 16 replicates for three times (total n = 48) on three different lots of the product.

The coefficients of variation (% CV) for the between and for the within assays were calculated.

From the OD450nm values obtained the following mean values have been derived:

| | NC | CAL | PC |
|---|---|---|---|
| **OD450nm** | 0.095 | 1.135 | 2.495 |
| **DEV.ST.** | 0.010 | 0.070 | 0.074 |
| **%CV** | 9.6 | 6.2 | 3.0 |

The variability shown in the table does not lead to any misinterpretation in particular of a sample closed to the diagnostic threshold of the assay.

**P. 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.

Following the international guidelines (CLSI C56-A) with samples containing fibrin particles or aggregates, false results can be obtained. It is recommended, in these cases, centrifugation of the samples before use

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**Symbols used on labels and packaging**

IVD = In vitro medical diagnostic device

REF = Catalog number

LOT = Lot number

 = Manufacturer

 = Expiration date

 = Storage Temperature

 = Instructions for use