Syphilis Ab One Version ULTRA

Enzyme Immunoassay for the determination of antibodies to Treponema Pallidum in serum and plasma

- for “in vitro” diagnostic use only -

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REF SIAB1ULTRA.CE
96/192/480/960 Tests

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A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the one-step qualitative determination of antibodies (IgG, IgM and IgA) to Treponema Pallidum.

The kit is intended for the screening of blood units and the follow-up of Tp-infected patients. For "in vitro" diagnostic use only.

B. INTRODUCTION

Syphilis is a sexually transmitted disease caused by Treponema pallidum or Tp, bacterium belonging to the family of Spirochaetaceae. Tp is gram negative and is considered strictly anaerobe, exhibiting a characteristic mobility due to periplasmic flagella. A cell wall and a cytoplasmic membrane enclose the cytoplasmatic content.

Syphilis is a complex, acute, chronic infectious disease with diverse clinical manifestations, depending upon the stage of infection and the individual response. The period of incubation ranges from 10 days to 3 months and antibodies are usually detected after 2-4 weeks from the primary lesion.

Many assays have been developed for the immunological detection of the T.pallidum infection in the past (VDRL, TPHA, RPR) still currently in use at the diagnostic laboratory.

Recently, ELISA techniques have been applied to syphilis antibody screening in blood banks and Infectious Disease Departments, allowing the clinicians to use automatic analysis instruments and optical reading records.

C. PRINCIPLE OF THE TEST

Microplates are coated with purified Treponema pallidum synthetic antigens (p15, p17 and p47).

Patient's serum/plasma is added to the microwell together with a mix of Tp synthetic antigens, labelled with peroxidase (HRP).

The specific immunocomplex, formed in the presence of anti Tp Ab in the sample, is captured by the solid phase.

At the end of the one-step incubation, microwells are washed to remove unbound serum proteins and HRP conjugate.

The chromogen/substrate is then added and, in the presence of captured immunocomplex, the colorless substrate is hydrolyzed by the bound HRP conjugate to a colored end-product. After blocking the enzymatic reaction, its optical density is measured by an ELISA reader.

The color intensity is proportional to the amount of anti Tp Ab present in the sample.

The version ULTRA is suitable for automated screenings.

D. COMPONENTS

The standard configuration of the kit contains sufficient reagents to perform 192 tests.

1. Microplate: MICROPLATE

n° 2 microplates. 12 strips of 8 breakable wells. Microplates are coated with purified Treponema pallidum synthetic antigens (p15, p17 and p47).

Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Negative Control CONTROL -

1x4.0ml/vial. Ready to use control. It contains 50% Goat Serum, 0.2 M Tris buffer, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. Yellow colour coded

3. Positive Control CONTROL +

1x4.0ml/vial. Ready to use control. It contains inactivated human serum positive to Tp, 5% BSA, 10 mM phosphate buffer pH 7.4+/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. Green colour coded

4. Calibrator: CAL …ml

N° 2 vials. Lyophilized calibrator. It contains inactivated anti Tp antibodies, calibrated against WHO 1st International Standard for human syphilitic plasma IgG & IgM NIBSC Code: 05/132, 4% Bovine serum albumin, 2% Mannitol, 50mM Tris buffer pH 7.8, 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300.

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

2x60ml/bottle. 20x concentrated solution containing 0.045% ProClin 300 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

1x25.0 ml/bottle. Ready-to-use solution. It contains Tp synthetic antigens, labelled with HRP, Tris buffer supplemented with 0.045% ProClin 300, Tween 20 and BSA . Red color coded

7. Chromogen/Substrate SUBS TMB

1x25ml/bottle.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 H2O2.

Note: To be stored protected from light as sensitive to strong illumination.

8. Sulphuric Acid: H2SO4 0.3 M

1x25ml/ bottle.Contains 0.3 M H_2SO_4 solution.

Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

9. Plate sealing foil n° 4

10. Package insert n° 1

Important note: Only upon specific request, Dia.Pro can supply reagents for 96, 480, 960 tests , as reported below **:**

Number of tests	96	480	960
Code	**SIAB1ULTRA.CE.96**	**SIAB1ULTRA.CE.480**	**SIAB1ULTRA.CE.960**
1.Microplate	n°1	n°5	n°10
2.NegativeControl	1x2.0ml/vial	1x10ml/vial	1x20ml/vial
3.PositiveControl	1x2.0ml/vial	1x10ml/vial	1x20ml/vial
4.Calibrator	n° 1 vial	n° 5 vials	n° 10 vials
5.Wash buff conc	1x60ml/bottle	5x60ml/bottles	4x150ml/bottles
6.Enz. Conjugate	1x16ml/vial	2x40ml/bottles	4x40ml/bottles
7.Chromog/Subs	1x16ml/vial	2x40ml/bottles	4x40ml/bottles
9.Sulphuric Acid	1x15ml/vial	2x40ml/bottles	2x80ml/bottles
10.Plate seal foils	n° 2	n° 10	n° 20
11.Pack. insert	n° 1	n° 1	n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (100ul) and disposable plastic tips.
2. EIA grade water (bidistilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 45 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 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. 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. Do not reuse disposable tips.
10. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
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. 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: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture 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. Haemolysed (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°…+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 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 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

Microplate:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned green, indicating a defect in conservation.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°-8°C.
After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Negative and Positive Controls:

Ready to use. Mix well on vortex before use.

Calibrator:

Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The calibrator after dissolution is not stable. Store frozen in aliquots at –20°C.*

Wash buffer concentrate:

The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: Once diluted, the wash solution is stable for 1 week at +2..8° C.

Enzyme conjugate:

Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

Chromogen/Substrate:

Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. 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 weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/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 $\pm$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 $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0;(d) repeatability $\geq$ 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..8°C, firmly capped.
8. Dia.Pro'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.

L. 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 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.

M. 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 an automatic workstation is used, first assure that the instrument is validated according to point I.6.
Than set the same procedure as in the Manual Assay accordingly with the operation of the automatic workstation.

Manual Assay:

1. Place the required number of microwells in the microwell holder. Store the other strips into the bag in presence of the desiccant at 2..8°C, sealed. Leave A1 well empty for the operation of blanking.

2. Dispense 100µl of Negative Control in triplicate, 100ul of Calibrator in duplicate and 100ul of Positive Control in single in proper wells, followed by 100ul of each of samples.
Do not dilute Controls and Calibrator as they are pre-diluted, ready to use!
Check for the presence of samples in wells by naked eye (there is a marked color difference between empty and full wells) or by reading at 450/620nm. (samples show OD values higher than 0.100).

3. Dispense 100ul Enzymatic Conjugate in all wells, except for A1, used for blanking operations.

Important note:

1. *Be careful not to touch the inner surface of the well with the pipette tip when the conjugate is dispensed. Contamination might occur.*
2. *Strips have to be sealed with the adhesive sealing foil only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*

4. Incubate the microplate for **45 min at 37°.**

5. Wash the microplate with an automatic washer as reported in section I.3.

6. Pipette 100 µl TMB/H_2O_2 mixture into each well, the blank well included. Check that the reagent has been correctly added.

7. **I**ncubate the microplate for **15 minutes** at **room temperature (18-24°C).**

Important note: *Do not expose to strong direct light as a high background might be generated.*

8. Pipette 100ul Sulphuric Acid into all the wells using the same pipetting sequence as in step 6 to stop the enzymatic reaction. Addition of acid will turn the positive control and positive samples from blue to yellow/brown.

9. Measure the color intensity of the solution in each well, asdescribed in section I.5, with a microplate reader at 450nm (reading) and at 620-630nm (background subtraction), blanking the instrument on A1 well (mandatory).

Important note:
~~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.
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.

N. ASSAY SCHEME

Method	Operations
Controls & Calibrator (*) Samples Enzyme Conjugate	100 ul 100 ul 100 ul
1st incubation	**45 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2	100 ul
2nd incubation	**15 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

Important notes:

- (*)*The Calibrator (CAL) does not affect the Cut Off calculation, therefore it does not affect the test's results calculation.*
- (*)*The Calibrator (CAL) used only if a laboratory internal quality control is required by the Management.*

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–Not Mandatory PC = Positive Control
S = Sample

O. INTERNAL QUALITY CONTROL
A check is carried out on the controls 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	< 0.050 OD450nm value
Negative Control (NC)	< 0.200 mean OD450nm value after blanking
Positive Control (PC)	> 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 > 0.050 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
Negative Control (NC) > 0.200 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.
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.

**** Note:**
If the Calibrator has used, verify the following data:

Check	Requirements
Calibrator (CAL)	S/Co > 1.1

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
Calibrator S/Co < 1.1	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (e.g.: dispensation of negative control instead of calibrator); 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.

Anyway, if all other parameters (Blank, Negative Control, Positive Control), match the established requirements, the test may be considered valid.

P. 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 + 0.200 = 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.*

Q. 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 Treponema Pallidum 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 of Tp 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 Tp antibodies (TPHA, VDRL), before a diagnosis of Tp infection is formulated.*
3. *When test results are transmitted from the laboratory to an informatics center, attention has to be done to avoid erroneous data transfer.*
4. *Diagnosis of Tp 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: 0.038 – 0.040 – 0.039 OD450nm
Mean Value: 0.039 OD450nm
Lower than 0.200 – Accepted

Positive Control: 2.589OD450nm
Higher than 1.000 – Accepted
Cut-Off = 0.080+0.200 = 0.280

Calibrator: 1.030 - 1.036 OD450nm
Mean value: 1.033 OD450nm S/Co = 3.7
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

R. PERFORMANCES
Evaluation of Performances has been conducted in accordance to what reported in the Internal Technical Specifications and following the *Centers for Disease Control and Prevention sexually Transmitted Diseases Treatment Guidelines,2002*.

R1. ANALYTICAL SENSITIVITY
The limit of detection of the assay has been calculated by means of the WHO 1st International Standard for human syphilitic plasma IgG & IgM NIBSC Code: 05/132.
The table below reports the results obtained for this material with three lots of products.
WHO was diluted in the Negative Control and examined in 4 replicates.

WHO 1st Int. Std. IU/ml	SIAB1ULTRA.CE Lot P1 OD450nm	SIAB1ULTRA.CE Lot P2 OD450nm
0.01	**0.889**	**0.903**
0.005	**0.487**	**0.521**
0.0025	**0.275**	**0.320**
0.00125	0.168	0.225
Negative Control	0.015	0.009

The product SIAB1ULTRA.CE shows an analytical sensitivity better than 0.0025 IU/ml.

R2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY
The Performance Evaluation of the device was carried out in a trial conducted on more than total 200 positive samples and more than 2000 negative samples.

R2.1 Diagnostic Specificity:
It is defined as the probability of the assay of scoring negative in the absence of specific analyte.
In addition to the first study, where a total of more 2000 samples, including unselected donors, hospitalized patients and potentially cross reacting specimens, were examined, the diagnostic specificity was recently assessed by testing a total of 1204 negative samples on three different lots. A value of specificity of 100% was found.
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 interference was observed if the sample is clear, particle free and not contaminated.

R2.2 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 200 specimens coming from Tp infection.
The diagnostic sensitivity was additionally evaluated on:

- The panel code PSS 901 supplied by Seracare;
- Two panels of European origin produced by EFS, France, and based on samples of European origin: lot # 08.150830, lot # 09/171002
- Syphilis Qualification Panel QSS701 supplied by Seracare
- BBI Diagnostics Accurun 156 Reagin (Syphilis) Positive Control supplied from Seracare

against a CE marked kit already present on the market.
A diagnostic sensitivity of 100% was found.

R3. PRECISION:
The Negative Control (NC), the Calibrator (CAL) and the Positive Control (PC) of the device were examined in 16 replicates for three run (total n = 48) on three different lots of the product.
The coefficients of variation (% CV) were calculated.
From the OD450nm values obtained the following mean values have been derived:

	NC	CAL	PC
OD450nm	0.030	1.138	3.319
DEV.ST.	0.005	0.072	0.086
CV%	14.3	6.4	2.6

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.

S. 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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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

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