Instructions for Use

Syphilis Ab Screen ELISA

IVD

CE

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Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida dell'inserto del pacco a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.

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1 INTENDED USE

Enzyme ImmunoAssay (ELISA) for the 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.

2 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.

3 PRINCIPLE OF THE TEST

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

The solid phase is first treated with the sample and anti Tp antibodies are captured, if present, by the antigens coated on the microplate.

After washing out all the other components of the sample, in the second incubation bound anti Tp total antibodies, are detected by the addition of Tp synthetic antigens, 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 Tp antibodies present in the sample. After blocking the enzymatic reaction, its optical density is measured by an ELISA reader.

The version is particularly suitable for automated screenings.

4 COMPONENTS

1. **Microplate**: MICROPLATE
   n° 1 microplate. 12 strips of 8 breakable wells.
   Microplate is coated with purified Treponema pallidum synthetic antigens (p15, p17 and p47).
   Plate is 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 -
   1 x 2.0 mL/vial. Ready to use control.
   It contains 5% BSA, 10 mM phosphate buffer pH 7.4 ±0.1, 0.09% sodium azide and 0.1% Kathon GC as preservatives. Yellow color coded
3. **Positive Control** CONTROL +
   1 x 2.0 mL/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 0.1% Kathon GC as preservatives. Green color coded
4. **Calibrator**: CAL …ml
   N° 1 vial. 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, 50 mM Tris buffer pH 7.8, 0.2 mg/mL gentamicine sulphate and 0.1% Kathon GC.
   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 16.0 mL/bottle. Ready-to-use solution.
   It contains Tp synthetic antigens, labelled with HRP, Tris buffer supplemented with 0.05% Kathon GC, Tween 20 and BSA. Red color coded
7. **Chromogen/Substrate** SUBS TMB
   1 x 16 mL/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 H_2O_2.
   Note: To be stored protected from light as sensitive to strong illumination.
8. **Sulphuric Acid**: H2SO4 0.3 M
   1 x 15 mL/ 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° 2
10. **Instructions for Use** n° 1

5 MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (100 µL) 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 450 nm (reading) and with 620-630 nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

6 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 °C - 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 hours 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.

7 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 °C - 8 °C for up to seven 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 0.2-0.8 µm filters to clean up the sample for testing.

8 PREPARATION OF COMPONENTS AND WARNINGS

Microplate:

Allow the microplate to reach room temperature (about 1 hour) before opening the container. Check that the desiccant has not turned green, indicating a defect in conservation.

In this case, call DRG's customer service.

Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at 2 °C - 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 lyophilized 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 °C - 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)

Legend:

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.

9 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 350 µL/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 450 nm and with a second filter (620-630 nm, strongly recommended) 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 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 station 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.

10 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 hour) 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 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.

11 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.

The Assay Procedure can be performed, with equal Performances, following two timing incubation protocols.

Chose the one required by your Country or Laboratory Official Regulations:

1. Long Incubation
   (1^{st} incubation 60 minutes, 2^{nd} and 3^{rd} incubations 30 minutes)
2. Short Incubation
   (1^{st}, 2^{nd} incubations 45 minutes and 3^{rd} incubations 15 minutes)

Automated assay:

In case an automatic workstation is used, first assure that the instrument is validated according to point 9.6.

Than set the same procedure as in the Manual Assay accordingly with the operation of the automatic workstation.

11.1 Long Incubation - 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 °C - 8 °C, sealed.
   Leave A1 well empty for the operation of blanking.
2. Dispense 100 µL of Negative Control in duplicate, 100 µL of Calibrator in duplicate and 100 µL of Positive Control in single in proper wells, followed by 100 µL 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 450/620 nm. (samples show OD values higher than 0.100).

Important note:
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.

3. Incubate the microplate for **60 min at 37 °C**.
4. Wash the microplate with an automatic washer as reported in section 9.3.
5. Pipette 100 µL Enzyme Conjugate into each well, except the first blanking well, and cover with the sealer.
   Check that this red colored component has been dispensed in all the wells, except A1.

Important notes:
Be careful not to touch the inner surface of the well with the pipette tip when the conjugate is dispensed. Contamination might occur.

6. Incubate the microplate for **30 min at 37 °C**.
7. Wash the microplate with an automatic washer as in step 4.
8. Pipette 100 µL TMB/H_2O_2 mixture into each well, the blank well included.
   Check that the reagent has been correctly added.
9. Incubate the microplate for **30 minutes at room temperature** (18 °C - 24 °C).

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

10. 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.
11. Measure the color intensity of the solution in each well, as described in section 9.5, with a microplate reader at 450 nm (reading) and at 620-630 nm (background subtraction, strongly recommended), blanking the instrument on A1 well.

11.2 Short Incubation - 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 °C - 8 °C, sealed.
   Leave A1 well empty for the operation of blanking.

2. Dispense 100 µL of Negative Control in duplicate, 100 µL of Calibrator in duplicate and 100 µL of Positive Control in single in proper wells, followed by 100 µL 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 450/620 nm. (samples show OD values higher than 0.100).

Important note:
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.

3. Incubate the microplate for **45 min at 37 °C**.
4. Wash the microplate with an automatic washer as reported in section 9.3.
5. Pipette 100 µL Enzyme Conjugate into each well, except the first blanking well, and cover with the sealer.
   Check that this red coloured component has been dispensed in all the wells, except A1.

Important notes:
Be careful not to touch the inner surface of the well with the pipette tip when the conjugate is dispensed. Contamination might occur.

6. Incubate the microplate for **45 min at +37 °C**.
7. Wash the microplate with an automatic washer as in step 4.
8. Pipette 100 µL TMB/H_2O_2 mixture into each well, the blank well included.
   Check that the reagent has been correctly added.
9. Incubate the microplate for **15 minutes at room temperature** (18 °C - 24 °C).

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

10. 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.
11. Measure the color intensity of the solution in each well, as described in section 9.5, with a microplate reader at 450 nm (reading) and at 620-630 nm (background subtraction, strongly recommended), blanking the instrument on A1 well.

Important notes:

If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450 nm. 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.

12 ASSAY SCHEME

Method	Operations (Long Incubation)	Operations (Short Incubation)
Controls & Calibrator (*) Samples 1st incubation	100 µL 100 µL 60 min	100 µL 100 µL 45 min
Temperature	+37 °C	+37 °C
Wash step	4-5 cycles	4-5 cycles
Enzyme conjugate 2nd incubation	100 µL 30 min	100 µL 45 min
Temperature	+37 °C	+37 °C
Wash step	4-5 cycles	4-5 cycles
TMB/H_2O_2 3rd incubation	100 µL 30 min	100 µL 15 min
Temperature	r.t.	r.t.
Sulphuric Acid	100 µL	100 µL
Reading OD	450 nm	450 nm

Important notes:
The Performances of the assays are not changed by the different incubation protocols.

- (*) 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	S3										
B	NC	S4										
C	NC	S5										
D	CAL(*)	S6										
E	CAL(*)	S7										
F	PC	S8										
G	S1	S9										
H	S2	S10										

Legenda:
BLK = Blank NC = Negative Control PC = Positive Control S = Sample CAL(*) = Calibrator–Not Mandatory

13 INTERNAL QUALITY CONTROL

A check is carried out on the controls any time the kit is used in order to verify whether their OD 450 nm values are as expected and reported in the table below.

Check	Requirements
Blank well	< 0.050 OD 450 nm value
Negative Control (NC)	< 0.200 mean OD 450 nm value after blanking
Positive Control (PC)	> 1.000 OD 450 nm 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 OD 450 nm	1. that the Chromogen/Substrate solution has not got contaminated during the assay
Negative Control (NC) > 0.200 OD 450 nm 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 OD 450 nm	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 OD 450 nm 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 don'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.

14 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 OD 450 nm 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.

15 INTERPRETATION OF RESULTS

Test results are interpreted as ratio of the sample OD 450 nm 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 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 centre, 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: 0.039 – 0.040 OD 450 nm
Mean Value: 0.039 OD 450 nm
Lower than 0.200 – Accepted

Positive Control: 2.589 OD 450 nm
Higher than 1.000 – Accepted
Cut-Off = 0.080 + 0.200 = 0.280

Calibrator: 1.030 - 1.036 OD 450 nm
Mean value: 1.033 OD 450 nm S/Co = 3.7
S/Co higher than 1.1 – Accepted

Sample 1: 0.070 OD 450 nm
Sample 2: 1.690 OD 450 nm
Sample 1 S/Co < 0.9 = negative
Sample 2 S/Co > 1.1 = positive

16 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*.

The Performances of the assay have been studied extensively using the two different Protocols (Long and Short) obtaining equivalent results.

16.1 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	EIA-4405 Lot 0313 OD 450 nm	EIA-4405 Lot 0313/2 OD 450 nm	EIA-4405 Lot 0313/3 OD 450 nm
0.01	1.149	1.240	1.046
0.005	0.576	0.574	0.506
0.0025	0.298	0.275	0.241
0.00125	0.141	0.143	0.123
Negative Control	0.016	0.017	0.024

The product EIA-4405 shows an analytical sensitivity better than 0.0025 IU/mL.

16.2 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.

16.2.1 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 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 2150 negative samples on four 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.

16.2.2 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 202(M) supplied by BBI, USA;
- 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.

16.3 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 OD 450 nm values obtained the following mean values have been derived:

	NC	CAL	PC
OD 450 nm	0.033	0.790	2.649
DEV.ST.	0.004	0.034	0.159
CV%	13.2	4.7	5.8

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.

17 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.

18 REFERENCES / LITERATURE

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SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
IVD	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Diagnostica in vitro	Diagnóstico in vitro	Diagnostic in vitro
REF	Catalogue number *	Artikelnummer *	No. di Cat.	No de catálogo	Référence
LOT	Batch code *	Chargencode *	Lotto no	Número de lote	No. de lot
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit *	Temperaturbegrenzung *	Temperatura di conservazione	Temperatura de conservacion	Température de conservation
	Use-by date *	Verwendbar bis *	Data di scadenza	Fecha de caducidad	Date limite d'utilisation
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Caution *	Achtung *			
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
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