AccuDiag™ Syphilis IgG/IgM ELISA Kit

REF 1465-P1

IVD See External Label 2-8°C 96 Tests

Syphilis IgG/IgM ELISA	
Principle	Indirect ELISA
Detection	Qualitative
Sample	20 µL serum/plasma
Incubation Time	75 minutes
Shelf Life	12 Months from the manufacturing date

*Read the package insert carefully and completely before performing the assay. Follow the instructions and do not modify them. Only by strict adherence to these instructions, the erroneous results can be avoided and the optimal performance of DAI Syphilis ELISA achieved.

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The Diagnostic Automation Inc. Syphilis ELISA is an enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of antibodies to Treponema Pallida in human serum or plasma specimens. It is in

SIGNIFICANCE AND SUMMARY

Syphilis is a disease caused by Spirochete bacterium called Treponema Pallidum (TP). If untreated, the organisms move throughout the body and can cause damage to many organs, making syphilis a life-threatening disease if not treated early enough. People who have been infected with Syphilis experience different symptoms during the 3 stages of the disease. Early, which is defined by the presence of the chancre at the site of inoculation. Syphilis may be further divided into primary, secondary, and early latent syphilis; late syphilis includes late latent and the various forms of tertiary Syphilis. The serological response to syphilis involves production of antibodies to a wide range of antigens, including non-specific antibodies and specific anti-TP antibodies. The first detectable response to infection is the production of specific antitreponemal IgM, which can be detected within 4 to 7 days after the chancre appears and until the end of the second week of infection; antitreponemal IgG appears aout four weeks later. By the time that symptoms develop, most patients have detectable IgG and IgM.

ASSAY PRINCIPLE

With DAI Syphilis ELISA, the detection of anti-TP antibodies is achieved by antigen "sandwich" enzyme-linked method (ELISA) where polystyrene microwell strips are pre-coated with recombinant Treponema Pallidum antigens expressed in E. coli. The specimen is incubated in the microwells together with recombinant TP antigens conjugated to horseradish peroxidase (HRP-Conjugate). The pre-coated antigens express the same epitopes as the HRP-Conjugate antigens, but are expressed in different hosts. In case of presence of anti-TP in the specimen, during incubation the pre-coated and conjugated antigens will be bound to the two variable doamins of the antibody and the specific antigens-antibody immunocomplex is captured on the solid phase. After washing to remove specimen and unbound conjugates, Chromogen solutions containing tetramethyl benzidine (TMB) and urea peroxide are added into the wells. In presence of the antigen-antibody-antigen "sandwich" complex, the colorless Chromogens are hydrolyzed by the bound HRP conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color can be measured and is proportional to the amount of antibody in the specimen. Wells containing specimens negative for anti-TP remain colorless.

SPECIMEN COLLECTION & PREPARATION

1. Specimen Collection: No special patient's preparation required. Collect the specimen in accordance with the normal laboratory practice. Either fresh serum or plasma specimens can be used with this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid haemolysis of the RBC. Care should be taken to ensure that the serum specimens are clear and not contaminated by microrganisms. Any visible particulate matters in the specimen should be removed by centrifugation at 3000 RPM (round per minutes) for 20 minutes at room temperature or by filtration.
2. Plasma specimens collected into EDTA, sodium citrate or herapin can be tested, but ***highly lipaemic, icteric, or hemolytic specimens should not be used*** as they can give false results in the assay. ***Do not heat inactivate specimens***. This can cause deterioration of the target analyte. Specimens with visible microbial contamination should never be used.
3. DAI Syphilis ELISA is intended ONLY for testing of individual serum or plasma specimens. Do not use the assay for testing of cadaver specimens, saliva, urine, or other body fluids, or pooled (mixed) blood.
4. Transportation and Storage: Store specimens at 2-8°C. Specimens not required for assaying within 1 week should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, specimens should be packaged and labeled in accordance with the

existing local and international regulations for transportation of clinical specimens and ethological agents.

REAGENTS

Materials provided with the kit

1. MICROWELL PLATE: (1x96 wells) Blank microwell strips fixed on white strip holder. The plate is sealed in aluminum pouch with desiccant. Each well contains recombinant TP antigens. The microwell strips can be broken to be used separately. Place unused wells or strips in the provided plastic sealable storage bag together with the desiccant and return to 2-8°C. Once opened, stable for 4 weeks at 2-8°C.
2. NEGATIVE CONTROL: (1x0.5ml per vial) Yellowish liquid filled in a vial with green screw cap. Protein-stabilized buffer tested non-reactive for anti-TP. Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
3. POSITIVE CONTROL: (1x0.5ml per vial) Red-colored liquid filled on a vial with red screw cap. Anti-TP antibodies diluted in protein-stabilized buffer. Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
4. HRP-CONJUGATE: (1x14ml per vial) Green-colored liquid in a white vial with red screw cap. Horseradish peroxides-conjugated recombinant TP antigens. Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
5. WASH BUFFER: (1x50ml per bottle) Colorless liquid in a clear bottle with white screw cap. Buffer solution containing surfactant. The concentrate must be diluted 1 to 20 with distilled/deionized water before use. Once diluted, stable stable for 1 week at room temperature, or for 2 weeks when stored at 2-8°C.
6. CHROMOGEN SOLUTION A: (1x8ml per vial) Colorless liquid filled in a white vial with green screw cap. Urea peroxide solution. Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
7. CHROMOGEN SOLUTION B: (1x8 ml per vial) Colorless liquid filled in a black vial with black screw cap. TMB (Tetramethylbenzidine), N,N-dimethylformamide. Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
8. STOP SOLUTION: (1x8ml per vial) Colorless liquid in a white vial with yellow screw cap. Diluted sulfuric acid solution (0.5M H_2SO_4). Ready to use as supplied. Once opened stable for 4 weeks at 2-8°C.
9. PLASTIC SEALABLE BAG: (1 unit) For enclosing the strips not in use.
10. PACKAGE INSERT: (1 copy)
11. CARDBOARD PLATE COVER: (2 sheets) To cover the plates during incubation and prevent evaporation or contamination of the wells.

Materials required but not provided

Freshly distilled or deionized water, disposable gloves and timer, appropriate waste containers for potentially contaminated materials, dispensing system and/or pipette, disposable pipette tips, absorbent tissue or clean towel, dry incubator or water bath, 37 ± 1°C, plate reader, single wavelength 450nm or dual wavelength 450/600~650nm, microwell aspiration/wash system.

ASSAY PROCEDURE

Reagents preparation: Allow the reagents to reach room temperature (18-30°C). Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed, resolubilize by warming at 37°C until crystals dissolve. Dilute the Wash buffer (20X) as indicated in the instructions for washing. Use distilled or deionized water and only clean vessels to dilute the buffer. All other reagents are **READY TO USE AS SUPPLIED**.

1. **Preparation:** Mark three wells as Negative central (e.g. B1, C1, D1), two wells as Positive control (e.g. E1, F1) and one Blank (eg. A1, neither specimens nor HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.
2. **Adding HRP:** Conjugate: Add 100µl of HRP-Conjugate into each well except the Blank
3. **Adding Specimen:** Add 20µl of Positive control, Negative control, and Specimen into their respective wells except the Blank. Note: Use a separate disposal pipette tip for each specimen, Negative Control, Positive Control to avoid cross-contamination. Mix by tapping the plate gently.
4. **Incubating:** Cover the plate with the plate cover and incubate at 37°C for 60 minutes
5. **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Wash Buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel, and tap it to remove any remainders.
6. **Coloring:** Add 50µl of Chromogen Solution A and then 50µl Chromogen Solution B into each well including the Blank, mix gently. Incubate the plate at 37°C for 15 minute5 avoiding light. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Positive control and anti-TP positive specimen wells.
7. **Stopping Reaction:** Using a multichannel pipette or manually, add 50µl of Stop Solution into each well and mix gently. Intensive yellow color develops in Positive control and anti-TP positive specimen wells.
8. **Measuring the Absorbance:** Calibrate the plate reader with the Blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at 600~650nm. Calculate the Cut-off value and evaluate the results. (Note: read the absorbance within 10 minutes after stopping the reaction).

INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential in order to obtain correct and precise analytical data.
2. It is therefore, recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles of 350-400µl/well are sufficient to avoid false positive reactions and high background.
3. To avoid cross-contaminations of the plate with specimen or HRP-conjugate, after incubation, do not discard the content of the wells but allow the plate washer to aspirate it automatically.
4. Assure that the microplate washer liquid dispensing channels are not blocked or contaminated and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to carry out 5 washing cycles, dispensing 350-400µl/well and aspirating the liquid 5 times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution at a final concentration of 2.5% for 24 hours, before they are wasted in an appropriate way.

The concentrated Wash buffer should be diluted 1:20 before use. If less than a whole plate is used, prepare the proportional volume of solution.

SUMMARY OF THE ASSAY PROCEDURE

Use this summary only as a reference and always follow the detailed method sheet when performing the assay.

Add HRP-Conjugate	100µl
Add specimen	20µl
Incubate	60 minutes

Wash	5 times
Coloring	50µl A + 50µl B
Incubate	15 minutes
Stop the reaction	50µl stop solution
Read the absorbance	450nm or 450/600~650nm

EXAMPLE SCHEME OF CONTROLS/SPECIMENS DISPENSING

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	S3										
B	Neg.	…										
C	Neg.	…										
D	Neg.											
E	Pos.											
F	Pos.											
G	S1											
H	S2											

RESULTS & QUALITY CONTROL

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each specimen absorbance (A) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well A value from the print report values of specimens and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well A value from the print report values of specimens and controls.

Calculation of the Cut-off value (C. O.) = Nc + 0.18
(Nc= the mean absorbance value for three negative controls)

Quality control (assay validation): The test results are valid if the Quality Control criteria are fulfilled. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient specimen being analyzed.

- The A value of the Blank well, which contains only Chromogen and Stop solution, is <0.080 at 450nm.
- The A values of the Positive control must be ≥0.800 at 450/600~650nm or at 450nm after blanking.
- The A values of the Negative control must be ≤0.100 at 450/600~650nm or at 450nm after blanking.

If one of the Negative control A values does not meet the Quality Control criteria, it should be discarded and the mean value calculated again using the remaining two values. If more than one Negative control A values do not meet the Quality Control Range specifications, the test is invalid and must be repeated.

Example:
1. Quality Control

Blank well A value=: A1 = 0.025 at 450nm (Note: blanking is required only when reading with single filter at 450nm)

Well No.:	B1	C1	D1
Negative control A values after blanking:	0.020	0.012	0.016
Well No.:	E1	F1	
Positive control A values after blanking:	2.421	2.369	

All control values are within the stated quality control range

2. Calculation of Nc: = $(0.020+0.012+0.016)/3$ = 0.016
3. Calculation of the Cut-off: (C.O.) = 0.016 + 0.18 = 0.196

INTERPRETATION

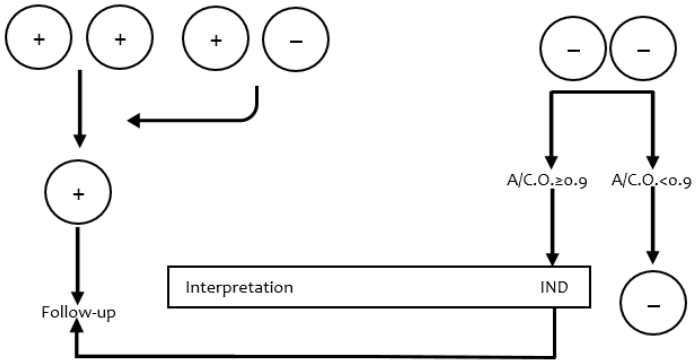
Negative Results (A / C.O. < 1): Specimens giving absorbance less than the Cut-off value are negative for this assay, which indicates that no anti-TP antibodies have been detected with DAI Syphilis ELISA, therefore there are no serological indications for current infection with TP.

Positive Results (A / C.O. ≥1): Specimens giving absorbance equal to or greater than the Cut-off value are considered initially reactive, which indicates that anti-TP antibodies have probably been detected using DAI Syphilis ELISA. All initially reactive specimens should be retested in duplicate using DAI Syphilis ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for antibodies to TP with DAI Syphilis ELISA.

Borderline (A / C.O. = 0.9-1.1): Specimens with absorbance to Ct-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicate is required to confirm the initial results.

Follow-up, confirmation and supplementary testing of any positive specimen with other analytical system is required. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.

INITIAL RESULTS INTERPRETATION AND FOLLOW-UP ALL INITIALLY REACTIVE OR BORDERLINE SPECIMENS



IND = non interpretable

- If, after retesting of the initially reactive specimens, both wells are negative results (A/C.o.<9), these specimens should be considered as non-repeatable positive (or false positive) and recorded as negative. As with many very sensitive ELISA assays, flase positive results can occur due to several reasons, most of which are connected with, but not limited to, inadequate washing step.
- If after tretesting in duplicate, one or both wells are positive results, the final result from this ELISA test should be recorded as repeatedly reactive. Repeatedly reactive specimens could be considered positive for antibodies to TP and therefore the patient is probably infected with TP.
- After retesting in duplicate, specimens with values close to the Cut-off value should be interpreted with caution and considered as "borderline" zone specimen, or uninterpretable for the time of testing.

PERFORMANCE CHARACTERISTICS

The clinical performances of this assay have been evaluated by a panel of specimens obtained from 4293 healthy blood donors from 8 blood banks. The sensitivity of DAI Syphilis ELISA of blood donors were 99.52% (413/415), while the specificity were 99.95% (3859/3861).

Testing Center	TPPA Positive	TPPA Suspicious	TPPA Negative
Blood Bank 1	14/14	–	2888/2890
Blood Bank 2	6/6	–	521/521
Blood Bank 3	76/76	4/6	59/59
Blood Bank 4	8/8	0/2	148/148
Blood Bank 5	90/90	1/1	13/13
Blood Bank 6	80/80	–	12/12
Blood Bank 7	2/2	0/2	148/148
Blood Bank 8	137/139	5/6	70/70
Total	413/415	–	3859/3861

The serum specimens collected from 222 syphilitic patients who had been diagnosed correctly by clinic (including 1st period, 2nd period, 3rd period and latent period Syphilis), 42 autoimmune diseases patients excluding syphilis (RPR testing positive) and 270 healthy blood donors, then they were tested for antibody against Treponema pallidum by RPR, TPPA and TP-ELISA methods respectively. Results: The positive rates of TP-ELISA, TPPA and RPR for detection of antibody against Treponema pallidum were 97.30% (216/222), 95.95% (213/222) and 90.54% (201/222) respectively. There was no statistical difference between TPPA and TP-ELISA for diagnose of syphilis (P>0.05). It didn't appear false positive in TPAA and TP-ELISA methods to 42 immune disease patients excluding syphilis.

Specimens		anti-TP ELISA		TRUST/RPR		TPAA/TPHA	
Syphilis	No.	+	–	+	–	+	–
1st period	66	60	6	55	11	59	7
2nd period	140	140	0	131	9	138	2
3rd period	2	2	0	2	0	2	0
Latent period	14	14	0	13	1	14	0
TOTAL	222	216	6	201	21	213	9

Analytical Specificity
No cross reactivity was observed with specimens from patients infected with HAV, HCV, HBV, HTLV, CMV, and HIV.
No interference was observed from rheumatoid factors up to 2000U/ml.
No high dose hook effect observed during clinical testing.
The assay performance characteristics are unaffected from elevated concentrations of bilirubin, hemoglobin, and triolein.

LIMITATIONS OF THE ASSAY

1. Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2. Antibodies may be undetectable during the early stage of the disease and in some immunosuppressed individuals. Therefore, negative results obtained with DAI Syphilis ELISA are only indication that the specimen does not contain detectable level of anti-TP antibodies and any negative result should not be considered as conclusive evidence that the individual is not infected with TP.
3. If, after retesting of the initially reactive specimens, the assay results are negative, these specimens should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step.
4. The most common assay mistakes are: using kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add specimens or reagents, improper operation with the laboratory equipment, timing errors, the use of highly hemolyzed specimens or specimens containing fibrin, incompletely clotted serum specimens.
5. The prevalence of the marker will affect the assay's predictive values.
6. This assay cannot be utilized to test pooled (mixed) serum or plasma. DAI Syphilis ELISA has been evaluated only with individual serum or plasma specimens.
7. DAI Syphilis ELISA is a qualitative assay and the results cannot be used to emasure antibody concentration.

STORAGE CONDITIONS

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8°C, do not freeze. To assure maximum performance of DAI Syphilis ELISA, during storage, protect the reagents from contamination with microorganism or chemicals.

PRECAUTIONS

TO BE USED ONLY BY QUALIFIED PROFESSIONALS
The ELISA assays are time and temperature sensitive. To avoid incorrect result, *strictly follow the test procedure steps and do no modify them.*

1. Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.
2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.
3. ***CAUTION – CRITICAL STEP: Allow the reagents and specimens to reach room temperature (18-30°C) before use. Shake reagent gently before use. Return at 2-8°C immediately after use.***
4. Use only sufficient volume of specimen as indicated in the procedure steps. Failure to do so, may cause low sensitivity of the assay.
5. Do not touch the exterior bottom of the wells; fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.
6. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.
7. Avoid long time interruptions of assay steps. Assure same working conditions for all wells.
8. Calibrate the pipette frequently to assure the accuracy of specimens/reagents dispensing. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations.
9. Assure that the incubation temperature is 37°C inside the incubator.
10. When adding specimens, do not touch the well's bottom with the pipette tip.
11. When measuring with a plate reader, determine the absorbance at 450nm or at 450/600-650nm.
12. The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.

13. If using fully automated equipment, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.
14. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.
15. ***WARNING: Materials from human origin may have been used in the preparation of the Negative Control of the kit. These materials have been tested with tests kits with accepted performance and found negative for HBsAg and antibodies to HIV 1/2., HCV, TP However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Bovine derived sera have been used for stabilizing of the positive and negative controls. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.***
16. Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.
17. Chemical should be handled and disposed of only in accordance with the current GLP (Good laboratory practice) and the local or national regulations.
18. The pipette tips, vials, strips and specimen containers should be collected and autoclaved for not less than 2 hours at 121°C or treated with 10% sod1um hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should NEVER be autoclaved. Materials Safety Data Sheet (MSDS) available upon request.
19. Some reagents may cause toxicity, irritation, burns or have carcinogenic effect as raw materials Contact with the skin and the mucosa should be avoided but not limited to the following reagents: Stop solution, the Chromogens, and the Wash buffer.
20. The Stop solution 0.5M H_2SO_4 is an acid. Use it with appropriate care. Wipe up spills immediately and was with warm water if come into contact with the skin or eyes.
21. ProClin™ 300 0.1% used as preservative, can cause sensation of the skin. Wipe up spills immediately and was with warm water if come into contact with the skin or eyes.

INDICATIONS OF STABILITY DETERIORATION OF THE REAGENT: Values of the Positive or Negative Controls, which are out of the indicated quality control range, are indicators of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the specimens must be retested. In case of constant erroneous results and proven deterioration or instability of the reagents, immediately substitute the reagents with new one or contact DAI technical support for further assistance.

REFERENCES

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

ISO 13485 Quality Management for Medical Devices CERTIFIED

Diagnostic Automation/Cortez Diagnostics, Inc.
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Date Adopted	**2023-11**
Brand Name	**AccuDiag™**
REF 1465-P1	**AccuDiag™ - Syphilis IgG/IgM ELISA**
EC REP	**CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands** www.cepartner4u.eu
Revision Date: 2016-09-18	