Size : 274 x 218 mm

TREPOLISA 3.0®

3rd Generation ELISA for Treponemal Antibodies

Third Generation Double Antigen Sandwich Enzyme Linked Immunosorbent Assay (ELISA) for the detection of Antibodies to *Treponema pallidum* in human serum or plasma in human serum or plasma

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

TREPOLISA® 3.0 is intended to be used for the detection of total antibodies (i.e., IgG, IgM, IgA, etc.) to *Treponema pallidum* in human serum or plasma

SUMMARY AND EXPLANATION

Syphilis is a sexually transmitted (venereal) disease caused by the spirochete *Treponema pallidum*. The disease can also be transmitted congenitally thereby attaining its importance in antenatal screening. After infection the host forms non-treponemal anti-lipoidal antibodies (reagins) to the lipoidal material released from the damaged host cells as well as treponema specific antibodies. Serological tests for non-treponemal antibodies such as VDRL, RPR, TRUST etc. are useful as screening tests. Tests for treponema specific antibodies such as TPHA, FTA-ABS, rapid treponema antibody tests and ELISA are gaining importance as screening as well as confirmatory tests because they detect the presence of antibodies specific to *Treponema pallidum*.

PRINCIPLE OF THE ASSAY

Microwell strips are coated with recombinant 47 Kd and 17 Kd antigens. The same antigens are conjugated to HRP. Samples along with positive and negative controls are added in the coated wells and incubated simultaneously with antigen HRP conjugate. The wells are washed to remove unbound components. Captured antibodies are detected by adding substrate. The reaction is stopped after specified time with acid and absorbance is determined for each well at 450 nm with an ELISA reader. The cutoff value is calculated by the given formula and absorbances of all the wells are compared with the cutoff value. Any sample having absorbance more than the cutoff value is considered reactive.

KIT COMPONENTS

TREPOLISA® 3.0 has following components:

1. Coated microwells : 96 microwells (12 x 8 wells) are coated with recombinant 47 Kd and 17 Kd antigens.
2. Positive control: Inactivated and stabilized human serum reactive for *Treponema pallidum* antibodies, non-reactive for HIV, HCV & HBsAg with preservatives.
3. Negative control: Inactivated and stabilized human serum non reactive for *Treponema pallidum*, HIV, HBsAg and HCV.
4. Conjugate: *Treponema pallidum* antigens-HRP conjugate (25X). To be diluted 25 times with conjugate diluent.
5. Conjugate diluent: Buffered solution containing stabilizing proteins and preservatives. Ready to use.
6. Substrate: Solution containing Tetramethyl benzidine (TMB) and hydrogen peroxide. Ready to use.
7. Wash buffer: Buffer contains surfactants (20 X). To be diluted 20 times with distilled or deionized water.
9. Stop solution:Diluted Sulphuric acid.
9. Microwell holder.
10. Instruction for use.
11. ELISA protocol sheet.
11. Plate sealer.

STORAGE AND STABILITY

(1). **TREPOLISA® 3.0** kit is stable at 2-8°C up to the expiry date printed on the label. (2).Coated microwells should be used within one month of opening the pouch. Each pouch contains 3 strips of 8 wells. Any unused well(s) should be resealed with cellotape (and desiccant pouch) to prevent moisture absorption and stored at 2-8°C for future use. (3). In case the desiccant pouch changes colour from blue to white, the strips should not be used.(4).Diluted conjugate must be used immediately. (5).

MATERIAL REQUIRED BUT NOT PROVIDED

1. Manual or automatic pipette.
2. Pipette tips.
3. Incubator.
4. Glass or polypropylene container for conjugate dilution.
5. Test tubes.
6. Absorbent sheets.
7. ELISA washer
8. ELISA reader.
9. Pipetting troughs or boats.
10. Disinfectant.
11. Reagent grade water
12. Disposable gloves.
13. Timer.
14. Biohazard waste container
15. Serological pipettes.

SAMPLE COLLECTION

(1). No prior preparation of the patient is required. (2). Collect blood specimen by venipuncture according to the standard procedure. (3).Serum or plasma can be used. (4). Specimen should be free of particulate matter and microbial contamination. (5). Preferably use fresh sample. However, specimen can be stored refrigerated for short duration. For long storage, freeze at -20°C

or below. Do not freeze samples in frost-free freezer. (6). Specimen should not be frozen and thawed repeatedly. (7). Do not heat inactivate before use. (8). Prior to use, specimen containing precipitate or particulate matter should be clarified by centrifugation.(9).Specimen containing sodium azide should not be used.

PRECAUTIONS

(1). Bring all reagents and specimen to room temperature before use. (2). Do not pipette any material by mouth. (3).Do not eat, drink or smoke in the area where testing is done. (4).Use protective clothing and wear gloves when handling samples. (5). Use absorbent sheet to cover the working area. (6). Immediately clean up any spills with sodium hypochlorite. (7).Dispose off all the reagents and material used as if they contain infectious agent. (8).Neutralize acid containing waste before adding hypochlorite. (9).Do not use kit after the expiration date. (10).Do not mix components of one kit with another. (11). Always use new tip for each specimen and reagent. (12). Do not let the dispensing tip of ELISA washer touch liquid in the wells. (13). Do not allow liquid from one well to mix with other wells. (14).Do not let the strips dry in between steps.

REAGENT PREPARATION

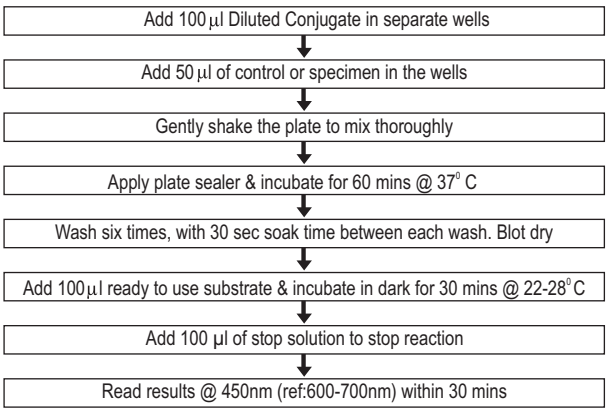
1. Dilute wash buffer 20 times (for example add 5 ml concentrated buffer to 95 ml distilled or deionized water).
2. Dilute conjugate 25 times (for example add 40 µl concentrated conjugate to 960µl conjugate diluent).

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
25 x Enzyme Conjugate	40µl	80µl	120µl	160µl	200µl	240µl	280µl	320µl	360µl	400µl	440µl	480µl
Conjugate Diluent	960µl	1920µl	2880µl	3840µl	4800µl	5750µl	6720µl	7680µl	8640µl	9600µl	10560µl	11520µl

TEST PROCEDURE

1. Bring all the reagents and specimen to room temperature before use.
2. Take out required number of strips and immediately close the pouch.
3. Prepare ELISA protocol sheet indicating the location of controls and specimen.
4. Use controls in duplicate.
5. Add **100µl** diluted conjugate in each well.
6. Add **50µl** of controls or specimen in separate wells.
7. Apply plate sealer and incubate for 60 minutes at 37°C.
8. Wash each well by filling approximately **350µl** diluted wash buffer, giving 30 seconds soak time for each wash and aspirating/flicking off six times. Blot dry.
9. Add **100µl** substrate in each well and incubate in dark at room temperature (22-28° C) away from light for 30 minutes.
10. Stop reaction by adding **100µl** stop solution. The stop solution should be added in the same sequence as substrate addition.
11. Read the absorbance at 450 nm with 600-700 nm as reference within 30 minutes of stopping the reaction.

TREPOLISA® 3.0 procedure flow-chart



RUN CRITERIA

1. The individual absorbance value of negative controls should be less than 0.1.
2. The individual absorbance value of positive controls should be more than 1.0.

CALCULATIONS

The cutoff value (COV) is calculated by adding 0.2 to average absorbance value of negative control.

EXAMPLE: COV = Av.Nc+0.2

Cutoff Value = Av.Nc +0.045 + 0.2 = 0.245

SAMPLE DATA

Well	Absorbance	Mean	Cutoff	Result
NC	0.040	0.045	0.245	
NC	0.050			
PC	2.680	2.715		
PC	2.750			
Sample 1	3.456			Reactive
Sample 2	0.537			Reactive
Sample 3	0.026			Non-Reactive

INTERPRETATION OF RESULTS

1. Samples with absorbance value less than the cut-off value are considered non-reactive by **TREPOLISA® 3.0** and are considered negative for treponemal antibodies.
2. Samples with absorbance value equal to or greater than cut-off value are considered reactive. The original sample should be retested in duplicate. Initially reactive sample that do not react in either of duplicates are considered negative for antibodies to *Treponema pallidum*. Initially reactive sample that reacts in either or both duplicates are considered repeatedly reactive.
3. If a sample is repeatedly reactive the probability of presence of antibodies to Treponema pallidum are high, especially with patients at high risk. Such samples should be retested with supplemental tests such as dark-field microscopy for confirmation.
4. In case of samples with high OD, there are possibilities of black precipitate formation after the addition of stop solution. This will not interfere with the interpretation of results.

PERFORMANCE CHARACTERISTICS

Eighty samples out of which three T. pallidum positive and seventy seven *T. pallidum* negative samples were tested with **TREPOLISA® 3.0** and compared with a commercially available 3rd generation *T. pallidum* positive ELISA kit. The results are given below:

Specimen Data	Total	TREPOLISA® 3.0	Commercial ELISA
Total Number	80	80	80
HIV Positive	3	3	3
HIV Negative	77	77	77

Sensitivity of **TREPOLISA® 3.0** : 100 % Specificity of **TREPOLISA® 3.0** : 100 %

Internal Evaluation I

Three hundred and twenty samples out of which fifty five *T. pallidum* positive (by TPHA) specimen and two hundred and sixty five *T. pallidum* negative (by TPHA) specimen were tested with **TREPOLISA® 3.0** and compared with 3rd generation commercially available T. *pallidum* ELISA. The results are given below:

Specimen data	Total	TREPOLISA®3.0	3rd Generation ELISA
Number of specimen tested	320	320	320
Number of *T. pallidum* Positive specimens	55	55	55
Number of *T. pallidum* Negative specimens	265	265	265

Based on this evaluation:
Sensitivity of **TREPOLISA® 3.0** : 100%
Specificity of **TREPOLISA® 3.0** : 100%

Precision

Inter & Intra-assay precision studies indicate that the CV is 10± 3%.

Internal Evaluation II

Evaluation with Mixed Titre Performance Panel
Results of Mixed Titre Performance Panel obtained from Boston Biomedica Inc., USA (PSS202) are given below:

PANEL ID	RPR TITER		ANTI-TREPONEMAL ANTIBODY ASSAY (S/CO)				
	BECTON-DICKINSON	WAMPOLE	DIESSE	TRINITY	3RD GEN. EIA	TPHA	TREPOLISA® 3.0
PSS202-01	128	128	> 5.5	3.3	7.61	NEGATIVE	15.50
PSS202-02	8	8	> 5.5	3.7	7.48	NEGATIVE	15.50
PSS202-03	4	4	> 5.5	2.2	7.39	POSITIVE	15.70
PSS202-04	NEGATIVE	NEGATIVE	0.3	0.2	0.22	NEGATIVE	0.01
PSS202-05	1	NEGATIVE	5.5	2.5	7.52	NEGATIVE	15.70
PSS202-06	64	128	> 5.5	4.6	7.50	POSITIVE	10.00
PSS202-07	8	8	> 5.5	3.5	7.50	POSITIVE	15.70
PSS202-08	1	1	> 5.5	0.2	7.50	POSITIVE	9.40

PANEL ID	RPR TITER		ANTI-TREPONEMAL ANTIBODY ASSAY (S/CO)				
	BECTON-DICKINSON	WAMPOLE	DIESSE	TRINITY	3RD GEN. EIA	TPHA	TREPOLISA® 3.0
PSS202-09	32	32	4.5	2.1	7.43	NEGATIVE	15.60
PSS202-10	16	64	> 5.5	3.8	7.50	NEGATIVE	15.70
PSS202-11	4	4	5.1	3.1	7.50	NEGATIVE	15.80
PSS202-12	32	64	> 5.5	2.0	7.44	POSITIVE	15.65
PSS202-13	1	2	> 5.5	3.1	7.40	POSITIVE	15.70
PSS202-14	2	2	> 5.5	3.7	7.44	POSITIVE	15.70
PSS202-15	32	64	> 5.5	2.2	7.50	NEGATIVE	15.70
PSS202-16	NEGATIVE	NEGATIVE	0.3	0.2	0.20	NEGATIVE	0.13
PSS202-17	2	2	> 5.5	2.3	7.40	NEGATIVE	15.30
PSS202-18	1	NEGATIVE	> 5.5	2.3	7.50	NEGATIVE	15.65
PSS202-19	2	2	> 5.5	3.5	7.45	NEGATIVE	15.70
PSS202-20	NEGATIVE	NEGATIVE	> 5.5	2.1	7.47	NEGATIVE	15.70

Note: Except TPHA, numerical values in Anti-treponemal antibody assays are expressed as specimen absorbance to cut off ratios (S/CO). Ratios more than or equal to 1.0 are considered positive. The data other than that for 3rd GEN. EIA, TPHA and **TREPOLISA®3.0** was supplied by BBI, USA.

From the above evaluation the following may be noted:

1. **TREPOLISA®3.0** shows good correlation with other anti-treponemal antibody assays.
2. TPHA is less sensitive than **TREPOLISA® 3.0** with some samples. Out of 17 positive samples in this panel only 7 has shown positive with TPHA.
3. There is average correlation with non-treponemal assay like RPR. Some samples from this panel have shown false negative with RPR. Therefore, the sensitivity of **TREPOLISA® 3.0** is higher than RPR in those samples.
4. The detectability of **TREPOLISA® 3.0** is higher than all anti-treponemal assays described here. This is indicated by the high S/CO, which is more than double (e.g. SAMPLE ID: PSS202-05; 2.5/7.52 Vs 15.70) for the same positive sample in **TREPOLISA® 3.0**.

LIMITATIONS OF THE TEST

(1) Though Trepolisa 3.0 is a reliable screening assay, it should not be used as a sole criterion for diagnosis of syphilis even if the sample is repeatedly reactive or has high absorbance value. Clinical diagnosis can be established only by a physician. (2) Trepolisa 3.0 should be used only as a screening test and its results should be confirmed by other supplemental methods before taking clinical decisions. (3) Absence of treponemal antibodies does not indicate that an individual is absolutely free of syphilis as the collection of sample and its timing vis-á-vis seroconversion and/or therapy will influence the test outcome. (4) Since various tests for syphilis differ in their antigenic composition, their reactivity patterns may differ. (5) It has been observed that most VDRL / RPR-positive samples also show positive with **TREPOLISA® 3.0**. However, since **TREPOLISA® 3.0** detects treponemal and non reagin antibodies, its results should not be compared with non-treponemal tests like VDRL, RPR, etc. (6) Testing of pooled samples is not recommended. (7) As with all diagnostic tests, a definitive clinical diagnosis should be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated

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(1) Clinical Diagnosis and Management by Laboratory Methods, John Bernard Henry, 17th Edition, 1979, W.B. Saunders Company. (2) Syphilis: New Diagnostic Directions, H. Young, International Journal of STD and AIDS, 1992, 3: 391-413. (3) Clinical Laboratory Diagnostics: Use and Assessment of Clinical Laboratory Results, Lothar Thomas, 1st Edition, 1998, TH-Books. (4) AABB Technical Manual, 13th Edition, 1999. (5) SI Egglestone and AJL Turner, Serological diagnosis of syphilis, Commun Dis Public Health 2000; 3: 158-62. (6) Schmidt BL et al. Comparative Evaluation of Nine Different Enzyme-Linked Immunosorbent Assays for Determination of Antibodies against Treponema pallidum in Patients with Primary Syphilis. Journal of Clinical Microbiology, March 2000, p. 1279-1282, Vol. 38, No. 3. (7) Castro R. et al. Evaluation of an enzyme immunoassay technique for detection of antibodies against *Treponema pallidum*, J. Clin. Microbiol. 2003, Jan; 41(1):250-3. (8) Data on file: Qualpro Diagnostics (P) Ltd.

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