Anti-T. pallidum IgG

3Z7611G
SM3Z7611G

CE

Rx Only

INTENDED USE

The Anti-T. pallidum IgG is intended for the qualitative detection of specific IgG class antibodies to *T. pallidum* in human serum. The test may be used in conjunction with non-treponemal testing and clinical finding to provide serological evidence of infection with *T. pallidum*. This test is for *In Vitro* diagnostic use only. This test is not intended for screening blood or plasma donors.

SIGNIFICANCE AND BACKGROUND

Treponema pallidum (subspecies pallidum) is a thin, gram-negative bacterium which belongs to the order Spirochaetales (1). It is one of the clinically important spirochetes and is thus related to such agents as *Borrellia burgdorferi* and *Leptospira. T. pallidum* is the etiologic agent of syphilis (1). There are no clinically available culture systems for *T. pallidum*, and microbiologic identification of the organism depends on techniques such as darkfield microscopy, direct fluorescent antibody stains, silver stains and serologic tests (2).

Syphilis occurs exclusively in humans. The vast majority of cases are acquired via sexual contact with an infected person. Other modes of acquisition include congenital transmission to the newborn and blood transfusion, but these are much less common (1, 2). Syphilis commonly presents in one of several stages: primary, secondary, latent, or tertiary syphilis. The methods for diagnosing early syphilis are dark field examination of active lesions and indirect fluorescent antibody tests. Serologic tests for syphilis include nontreponemal tests (VDRL) and treponemal FTAs or EIAs (1, 3). Both treponemal and nontreponemal tests are generally necessary to presumptively diagnose primary syphilis. As a rule, the treponemal tests stay positive for life following the initial infection, whether or not appropriate therapy has been administered (1, 3). The Anti-T. pallidum IgG Test System is intended to detect IgG class antibodies to *T. pallidum* in human sera.

PRINCIPLE OF THE ASSAY

The Anti-T. pallidum IgG is designed to detect IgG class antibodies to p17 antigen from *T. pallidum* in human sera. Wells of plastic microwell strips are sensitized by passive adsorption with *T. pallidum* antigen. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase Conjugated goat anti-human IgG is added to the wells and the plate is incubated. The Conjugate will react with IgG antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted Conjugate.
3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped, and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

TEST SYSTEM COMPONENTS

Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE:** The following components contain Sodium Azide as a preservative at a concentration of <0.1% (w/v): Controls, Calibrator, and SAVe Diluent®.

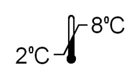
Kit Component	Quantity 96	Description
PLATE	1	Plate: 96 wells configured in twelve, 1x8-well, strips coated with recombinant p17 Treponema pallidum antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
CONJ	1	Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgG (Fc chain specific). 15mL, white-capped bottle. Ready to use.
CTRL +	1	Positive Control (Human Serum): 0.35mL, red-capped vial. 21X concentrate.
CAL	1	Calibrator (Human Serum): 0.5mL, blue-capped vial. 21X concentrate.
CTRL -	1	Negative Control (Human Serum): 0.35mL, green-capped vial. 21X concentrate.
DIL SPE	1	SAVe Diluent®: 30mL, green-capped, bottle containing Tween-20, bovine serum albumin and phosphate-buffered-saline, (pH 7.2 ± 0.2). Ready to use. **NOTE: The SAVe Diluent® will change color when combined with serum.**
SOLN TMB	1	TMB: 15mL, amber-capped, amber bottle containing 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use.
SOLN STOP	1	Stop Solution: 15mL, red-capped bottle containing 1M H_2SO_4, 0.7M HCl. Ready to use.
WASH 10X	1	Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. 100mL, clear-capped bottle containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). **NOTE: 1X solution will have a pH of 7.2 ± 0.2.**

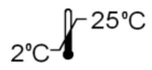
NOTE: The following components are not Test System Lot Number dependent and may be used interchangeably with the ZEUS ELISA Test Systems: TMB, Stop Solution, and Wash Buffer. SAVe Diluent® may be used interchangeably with any ZEUS ELISA Test System utilizing Product No. 005CC.

MATERIALS REQUIRED BUT NOT PROVIDED

1. ELISA microwell reader capable of reading at a wavelength of 450nm. **NOTE: Use of a single (450nm), or dual (450/620 - 650nm), wavelength reader is acceptable. Dual wavelength is preferred, as the additional reference filter has been determined to reduce potential interference from anomalies that may absorb light.**
2. Pipettes capable of accurately delivering 10 - 200µL.
3. Multichannel pipette capable of accurately delivering 50 - 200µL.
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or microwell washing system.
6. Distilled or deionized water.
7. One-liter graduated cylinder.
8. Serological pipettes.
9. Disposable pipette tips.
10. Paper towels.
11. Laboratory timer to monitor incubation steps.
12. Disposal basin and disinfectant (i.e., 10% household bleach - 0.5% sodium hypochlorite).

STORAGE CONDITIONS

	Coated Microwell Strips: Immediately reseal extra strips with desiccant and return to proper storage. After opening, strips are stable for 60 days, as long as the indicator strips on the desiccant pouch remain blue.
	Conjugate – DO NOT FREEZE.
	Unopened Kit, Calibrator, Positive Control, Negative Control, TMB, Sample Diluent.

	Stop Solution: 2 – 25 °C Wash Buffer (1X): 20 - 25°C for up to 7 days, 2 - 8°C for 30 days Wash Buffer (10X): 2 - 25°C

PRECAUTIONS

1. For *In Vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, consider the strips **potentially biohazardous materials** and handle accordingly.
4. The Controls are **potentially biohazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": Current Edition; and OSHA's Standard for Bloodborne Pathogens (5).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay**. Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The SAVe Diluent®, Controls, and Calibrator contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions upon hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
8. The Stop Solution is TOXIC if inhaled, has contact with skin or if swallowed. It can cause burns. In case of accident or ill feelings, seek medical advice immediately.
9. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
11. Wipe the bottom of the plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Do not use reagents from other sources or manufacturers.
14. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with Conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
25. Do not allow the Conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing Sodium Azide as a preservative. Residual amounts of Sodium Azide may destroy the Conjugate's enzymatic activity.
26. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.

SPECIMEN COLLECTION

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Infectious Disease (Current Edition).
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, consider all blood derivatives potentially infectious.
3. Use only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures in this assay (7, 8). Do not use if there are any added anticoagulants or preservatives. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2 - 8°C, for no longer than 48 hours. If a delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (9).

ASSAY PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20 - 25°C).
2. Determine the number of microwells needed. Allow for six Control/Calibrator determinations (one Reagent Blank, one Negative Control, three Calibrators and one Positive Control) per run. Run a Reagent Blank on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2 - 8°C.

EXAMPLE PLATE SET-UP		
	1	2
A	Blank	Patient 3
B	Negative Control	Patient 4
C	Calibrator	Etc.
D	Calibrator	
E	Calibrator	
F	Positive Control	
G	Patient 1	
H	Patient 2	

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of SAVe Diluent®) of the Negative Control, Calibrator, Positive Control, and each patient serum. **NOTE: The SAVe Diluent® will undergo a color change confirming that the specimen has been combined with the diluent.**
4. To individual wells, add 100µL of each diluted Control, Calibrator and patient specimen. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100µL of SAVe Diluent® to well A1 as a Reagent Blank. Check software and reader requirements for the correct Reagent Blank well configuration.
6. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
7. Wash the microwell strips 5 times.
    a. **Manual Wash Procedure**:
        1. Vigorously shake out the liquid from the wells.
        2. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
        3. Repeat steps 1. and 2. for a total of 5 washes.
        4. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with disinfectant at the end of the day's run.

    b. **Automated Wash Procedure**:
    If using an automated microwell wash system, set the dispensing volume to 300 - 350µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.

8. Add 100µL of the Conjugate to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
9. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100µL of TMB to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
12. Incubate the plate at room temperature (20 - 25°C) for 10 - 15 minutes.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including the Reagent Blank well, at the same rate and in the same order as the TMB. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the Reagent Blank. Read the plate within 30 minutes of the addition of the Stop Solution.

ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21.
2. Add diluted sample to microwell - 100µL/well.
3. ⟶ *Incubate 25 ± 5 minutes.*
4. Wash.
5. Add Conjugate - 100µL/well.
6. ⟶ *Incubate 25 ± 5 minutes.*
7. Wash.
8. Add TMB - 100µL/well.
9. ⟶ *Incubate 10 - 15 minutes.*
10. Add Stop Solution - 50µL/well - Mix.
11. READ within 30 minutes.

QUALITY CONTROL

1. Each time the assay is performed, the Calibrator must be run in triplicate. A Reagent Blank, Negative Control, and Positive Control must also be included.
2. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.
3. The mean OD value for the Calibrator, Positive Control, and Negative Control should fall within the following ranges:

	OD Range
Negative Control	≤0.250
Calibrator	≥0.300
Positive Control	≥0.500

   a. The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤0.9.
   b. The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥1.25.
   c. If the above conditions are not met the test should be considered invalid and should be repeated.
4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure, but will not ensure precision at the assay Cutoff.
5. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
6. Refer to CLSI document C24: Statistical Quality Control for Quantitative Measurement Procedures for guidance on appropriate QC practices.

INTERPRETATION OF RESULTS

1. **Calculations:**
   a. *Correction Factor*: The manufacturer determined a Cutoff OD Value for positive samples and correlated it to the Calibrator. The Correction Factor (CF) allows for the determination of the Cutoff Value for positive samples. It will also correct for slight day-to-day variations in test results. The Correction Factor is determined for each lot of components and is printed on the Component Label located in the Test System box.
   b. *Cutoff OD Value*: To obtain the Cutoff OD Value, multiply the CF by the mean OD of the Calibrator determined above.
   (CF x Mean OD of Calibrator = Cutoff OD Value)

c. *Index Values/OD Ratios*: Calculate the Index Value/OD Ratio for each specimen by dividing its OD Value by the Cutoff OD from step b.

Example:	Mean OD of Calibrator	=	0.793
	Correction Factor (CF)	=	0.25
	Cutoff OD	=	0.793 x 0.25 = 0.198
	Unknown Specimen OD	=	0.432
	Specimen Index Value/OD Ratio	=	0.432/0.198 = 2.18

2. **Interpretations:** Index Values/OD Ratios are interpreted as follows.

	Index Value/OD Ratio
Negative Specimens	≤0.90
Equivocal Specimens	0.91 to 1.09
Positive Specimens	≥1.10

a. An OD Ratio less than or equal to 0.90 indicates no detectable antibody to *T. pallidum* and should be reported as non-reactive for IgG antibody to *T. pallidum*.

b. Retest specimens with OD Ratio Values in the equivocal range (0.91 – 1.09) in duplicate. Report any two of the three results which agree. Evaluate repeatedly equivocal specimens using an alternate serological method and/or re-evaluate by drawing another sample one to three weeks later.

c. An OD Ratio greater than or equal to 1.10 is positive for IgG antibody to *T. pallidum,* the causative agent for syphilis. A positive test result presumes a current or past infection with *T. pallidum*. Report as reactive for IgG antibody to *T. pallidum*. Perform other *T. pallidum* serology assays to confirm or rule out a current case of active syphilis.

Table 1: *T. pallidum* Result Interpretation

Non-Treponemal Result	Treponemal Result	Report/Interpretation for All Excluding Neonates, Infants and HIV-infected Individuals*
Non-Reactive	Negative/Non-Reactive	No serologic evidence of infection with *T. pallidum* (incubating or early primary syphilis infection cannot be excluded).
Reactive	Negative/Non-Reactive	Current infection unlikely; probability of BFP secondary to other medical conditions (febrile diseases, immunizations, IVDU, autoimmune diseases, etc.). Recommend repeat testing (nontreponemal and treponemal by other test methods).
Non-Reactive	Positive/Reactive	Probable past infection or potential cross-reactivity with other spirochetes/related antigens; Recommend additional testing appropriate to clinical findings/history**; Possibility of false negative nontreponemal result due to prozone or late latent syphilis or neurosyphilis.
Reactive	Positive/Reactive	Presumptive evidence of current infection (or inadequately treated infection, persistent infection, reinfection or BFP if prior history); recommend additional testing consistent with clinical assessment. *
Non-Reactive	Not Done	Current infection unlikely; effectively treated infection if previously diagnosed and treated; cannot exclude incubating or early primary syphilis; cannot exclude latent or neurosyphilis.
Not Done	Negative/Non-Reactive	Current or past infection unlikely; cannot exclude incubating or primary syphilis.

*HIV-infected individuals may have delayed seroreactivity or negative serology.

**Quantitative nontreponemal testing; clinical history; repeated (sequential) serological testing for changes in titer.

LIMITATIONS OF THE ASSAY

1. Interpret test results in conjunction with the clinical evaluation and the results of other diagnostic procedures.
2. Performance characteristics of this device have not been established with syphilis-associated disease.
3. Hemolytic, icteric, or lipemic samples may interfere with the outcome of this assay. Avoid the use of these types of specimens.
4. Test results of specimens from immunosuppressed patients may be difficult to interpret.
5. Performance characteristics of this device have not been established for matrices other than serum.
6. Performance characteristics of this device have not been established with specimens containing heterophile antibodies which are known to cause false positive results in various immunoassays.

7. A positive result does not establish a diagnosis of Syphilis. Such a result may reflect a previous infection; a negative result can exclude a diagnosis of Syphilis except for incubating or early primary disease.

EXPECTED RESULTS

1. **Demographics And Age Distribution of The Intended Use Populations:**
   Nine hundred and ninety-eight (998) unselected samples, 500 each from individuals with a syphilis test requested and 498 from pregnant women with a syphilis test requested were tested (two samples were excluded from calculations due to questionable age). Site One, the manufacturer, located in the Northeast, tested a total of 200 samples, 100 from pregnant women and 100 from patients that had a syphilis test ordered. Site Two, a hospital laboratory located in the Northeast tested 400 samples; 200 from pregnant women and 200 from patients that had a syphilis test ordered. Site Three, a hospital laboratory located in the Mid-Atlantic region of the United States tested 400 samples; 200 from pregnant women and 200 from patients that had a syphilis test ordered. The patient demographics are summarized in Table 2.

Table 2: Demographics for Populations Tested

Populations	Number Tested	Mean	Median	Minimum	Maximum
Samples with Syphilis Test Ordered	500	34.2	31	5	88
Prospective Pregnant Women	500	28.8	28	15	48

2. **Expected Values/Reference Ranges:**
   To determine expected values in the populations tested, internal and external investigators assessed the device's performance with 500 masked samples prospectively collected from individuals and 498 samples from pregnant women. The samples were requested to be random, unselected sera submitted for syphilis antibody testing. Additional studies were conducted in a population of 1000 unselected hospitalized patients (one sample was QNS for testing). In the 500 prospectively collected samples from patients ranging in age from <1 to >70 years of age; seven tested positive. Four of the seven samples were from male patients for an observed prevalence of 57.1%; three of the seven samples were from females for an observed prevalence of 42.9%. The overall observed prevalence in this group was 1.4% (7/55 samples). In the 498 samples collected from pregnant women ranging in age from 15 to 48, three of 498 samples tested positive. The observed prevalence in this group was 0.6%. In the group of 999 samples from unselected hospitalized patients ranging in age from <1 to >70 years of age; 32 tested positive. Fourteen of the 32 samples were from male patients for an observed prevalence of 43.8%; 18 of the 32 samples were from females for an observed prevalence of 56.3%. The overall observed prevalence in this group was 3.2% (32/999 samples).

PERFORMANCE CHARACTERISTICS

1. **Comparative Study:**
   The Anti-T. pallidum IgGTest System was compared to a commercially marketed ELISA procedure for detection of IgG antibodies to *T. pallidum*. The testing sites, populations and amounts of samples that were tested follow in Table 3:

Table 3:	**Prospective Samples**			**Retrospective Samples**			
Site	**Patients with Syphilis Test Ordered**	**Pregnant Women with Syphilis Test Ordered**	**Unselected Hospitalized Patients**	**Purchased Sera from HIV Positive Patients**	**Purchased Pregnant Women Sera**	**Purchased Sera Requested to be RPR/TPPA +**	**CDC Syphilis Characterized Samples**
ZEUS	100	100	350	223	0	0	0
Northeast Hospital Lab	200	200	350	0	277	280	0
Mid-Atlantic Hospital Lab	200	200	300	0	0	0	0
CDC	0	0	0	0	0	0	157
Total	500	500	1000	223	277	280	157

   a. **Performance in Prospectively Collected Intended Use Populations:** The comparative study for the Intended Use Populations consisted of 500 unselected serum samples from patients with a syphilis test ordered and 500 purchased serum samples from pregnant women.

Table 4: Banked Sera From Patients with Syphilis Test Ordered

		Predicate					
		Positive	**Equivocal**	**Negative**	**Site Total**	**PPA/NPA**	**95% CI**
Anti-T. pallidum IgG	**Positive**	4	0	3	7	80.0	28.4 - 99.5
	Equivocal	1	0	1	2		
	Negative	0	0	491	491	99.2	97.9 - 99.8
	Site Total	5	0	495	500		

Table 5: Banked Purchased Sera from Pregnant Women with Syphilis Test Ordered

		Predicate					
		Positive	**Equivocal**	**Negative**	**Site Total**	**PPA/NPA**	**95% CI**
Anti-T. pallidum IgG	**Positive**	3	0	0	3	75.0	19.4 - 99.4
	Equivocal	0	0	0	0		
	Negative	1	0	494	495	100.0	99.4 - 100
	Site Total	4	0	494	498		

*Two samples did not meet the inclusion criteria for testing.

Performance characteristics for 1000 unselected hospitalized patients appear in Table 6. These samples were pulled from the routine chemistry workload of a hospital laboratory.

Table 6: Unselected Hospitalized Patients

		Predicate					
		Positive	**Equivocal**	**Negative**	**Site Total**	**PPA/NPA**	**95% CI**
Anti-T. pallidum IgG	**Positive**	13	1	18	32	61.9	38.4 - 81.9
	Equivocal	1	0	9	10		
	Negative	7	0	950	957	97.1	95.9 - 98.1
	QNS	0	0	1	1		
	Site Total	21	1	978	1000		

b. **Performance In Retrospectively Collected Special Populations:** Comparative studies for Special Populations were conducted at ZEUS Scientific. The samples were purchased from a serum vendor.

Table 7: Banked Purchased Known HIV-1 Positive Serum Samples

		Predicate					
		Positive	**Equivocal**	**Negative**	**Site Total**	**PPA/NPA**	**95% CI**
Anti-T. pallidum IgG	**Positive**	41	0	1	42	85.4	72.2 - 93.9
	Equivocal	1	0	0	1		
	Negative	4	2	174	180	99.4	96.9 - 100
	Site Total	46	2	175	223		

Table 8: Banked Purchased Sera from Pregnant Women Requested to be TPPA Positive (27) and RPR/TPPA Non-Reactive (250)

		Predicate					
		Positive	**Equivocal**	**Negative**	**Site Total**	**PPA/NPA**	**95% CI**
Anti-T. pallidum IgG	**Positive**	26	1	0	27	92.9	76.5 - 99.1
	Equivocal	0	0	0	0		
	Negative	2	0	248	250	99.6	97.8 - 100
	Site Total	28	1	248	277		

Table 9: Banked Purchased Sera Requested to be RPR/TPPA Reactive

		Predicate					
		Positive	**Equivocal**	**Negative**	**Site Total**	**PPA/NPA**	**95% CI**
Anti-T. pallidum IgG	**Positive**	259	1	4	264	98.5	96.2 - 99.6
	Equivocal	1			1		
	Negative	3		12	15	70.6	46.9 - 98.7
	Site Total	263	1	16	280		

A panel of 157 clinically characterized samples was obtained from the CDC and tested. The results are presented to convey further information on the performance of the Test System and do not imply endorsement of the assay by the CDC. The summary of performance with the characterized serum panel appears in Table 10.

Table 10: Performance with Clinically Characterized Samples

Clinical Diagnosis	Anti-T. pallidum IgG Results				% Agreement with Clinical Diagnosis Presented with 95% CI	
	Positive	Equivocal	Negative	Total		
Primary Treated	11	0	0	11	100 (11/11)	76.2 - 100
Secondary Untreated	41	0	2	43	95.3 (41/43)	84.2 - 99.4
Secondary Treated	39	0	0	39	100 (39/39)	92.6 - 100
Latent Untreated	6	0	5	11	54.5 (6/11)	23.4 - 83.3
Latent Treated	48	0	2	50	96.0 (48/50)	86.3 - 99.5
Congenital	1	1	1	3	33.3 (1/3)	0.84 - 90.6
Total	146	1	10	157	93.0 (146/157)	87.8 - 96.5

2. **Reproducibility:**
Reproducibility was evaluated internally and at two external clinical sites. The study was conducted as follows: 15 samples were identified and/or prepared by ZEUS Scientific for use in the study based upon their activity on the assay. Three samples each were selected that were negative, high negative, near cutoff, low positive and high positive. To assess reproducibility, on each day of testing, each sample was diluted twice, and each dilution was run in triplicate. This process was repeated by a second technologist resulting in twelve results per day. This was repeated for five days at each site and the resulting data used to assess reproducibility.

Table 11: Summary Of Multi-Site Reproducibility - Anti-T. pallidum IgG

Panel Member	Sample (N)	Mean Index Value	Within-Run		Within -Day		Between-Run		Between-Site		Total	
			StD	% CV	StD	% CV	StD	% CV	StD	% CV	StD	% CV
Negative 1	180	0.06	0.01	14.7	0.01	19.3	0.01	10.7	0.02	29.1	0.02	36.6
Negative 2	180	0.08	0.01	9.7	0.01	12.2	0.01	8.3	0.01	14.6	0.01	15.5
Negative 3	180	0.31	0.03	8.6	0.03	10.7	0.02	6.6	0.04	12.7	0.04	13.3
High Negative 1	180	0.80	0.04	5.1	0.05	6.3	0.03	4.2	0.06	7.3	0.06	7.3
High Negative 2	180	0.74	0.04	5.1	0.04	5.8	0.02	3.0	0.05	7.0	0.05	7.4
High Negative 3	180	0.76	0.04	5.0	0.04	5.6	0.21	2.7	0.05	6.5	0.05	7.2
Borderline 1	180	1.05	0.07	6.3	0.07	7.3	0.03	3.6	0.09	9.0	0.11	10.3
Borderline 2	180	1.13	0.05	4.7	0.06	5.4	0.04	3.1	0.07	6.0	0.08	6.7
Borderline 3	180	0.95	0.05	5.6	0.07	6.7	0.04	4.0	0.08	8.5	0.09	9.9
Low Positive 1	180	1.45	0.09	6.2	0.11	7.6	0.06	4.4	0.13	8.9	0.14	9.6
Low Positive 2	180	1.77	0.11	5.9	0.14	7.8	0.10	5.8	0.15	8.3	0.16	9.2
Low Positive 3	180	1.93	0.14	7.1	0.17	8.9	0.12	5.9	0.19	9.7	0.21	10.7
Positive	180	3.6	0.20	5.7	0.22	6.2	0.10	2.8	0.30	8.4	0.37	10.2
Positive 2	180	3.1	0.20	6.2	0.22	7.3	0.13	4.4	0.28	9.0	0.34	10.9
Positive 3	180	3.1	0.18	5.7	0.22	6.9	0.16	4.9	0.26	8.4	0.31	10.2
Non-Reactive Control	180	0.09	0.01	10.6	0.01	12.8	0.01	6.2	0.01	15.9	0.02	21.0
Reactive Control	180	3.9	0.16	4.1	0.12	5.1	0.14	3.5	0.22	5.7	0.02	5.9

Precision was evaluated internally at the manufacturer's site. The study was conducted as follows: 15 samples were identified and/or prepared by ZEUS Scientific for use in the study based upon their activity on the assay. Three samples each were selected that were negative, high negative, near cut-off, low positive and high positive. To assess precision, on each day of testing, each sample was diluted twice and tested. This was repeated by a second technologist in a separate run and resulted in four results per day. This was repeated for twelve days, and the resulting data used to assess precision.

Table 12: Summary of In-House Repeatability- Anti-T. pallidum IgG

Panel Member	Sample N	Mean AU/mL	Within-Run		Within -Day		Between-Run		Total	
			StD	% CV	StD	% CV	StD	% CV	StD	% CV
Negative 1	48	0.08	0.003	3.8	0.006	8.0	0.006	6.8	0.011	13.1

Negative 2	48	0.12	0.005	4.0	0.003	6.5	0.009	7.0	0.013	10.5
Negative 3	48	0.50	0.017	3.6	0.034	9.5	0.020	3.9	0.045	9.0
High Negative 1	48	0.75	0.057	7.6	0.030	8.6	0.019	2.5	0.058	7.7
High Negative 2	48	0.72	0.046	6.4	0.014	7.1	0.015	2.0	0.052	7.2
High Negative 3	48	0.74	0.015	2.0	0.018	4.7	0.011	1.4	0.034	4.5
Near Cut-off 1	48	0.92	0.028	3.0	0.025	5.0	0.036	3.9	0.056	6.1
Near Cut-off 2	48	1.04	0.022	2.1	0.014	3.9	0.033	3.1	0.045	4.3
Near Cut-off 3	48	0.95	0.037	3.9	0.025	6.4	0.036	3.8	0.061	6.4
Low Positive 1	48	1.48	0.029	2.0	0.029	3.9	0.014	0.9	0.058	3.9
Low Positive 2	48	1.43	0.026	1.8	0.020	2.5	0.017	1.2	0.050	3.5
Low Positive 3	48	1.65	0.027	1.6	0.037	4.2	0.018	1.1	0.078	4.7
High Positive 1	48	5.43	0.131	2.4	0.154	3.8	0.27	5.0	0.38	7.0
High Positive 2	48	4.85	0.110	2.3	0.176	3.6	0.17	3.6	0.29	6.0
High Positive 3	48	4.74	0.136	2.8	0.189	4.9	0.17	3.5	4.74	5.2
Non-Reactive Control	48	0.13	0.004	3.3	0.008	6.23	0.007	5.39	0.010	8.2
Reactive Control 1	48	5.63	0.049	7.5	5.5	5.15	0.29	5.11	0.42	7.51

3. **Cross Reactivity:**
Studies were performed at the manufacturing facility to assess cross reactivity with the Anti-T. pallidum IgG Test System using samples that were sero-positive to EBV, ANA, RF IgM, Rubella, HIV, HSV 1, HSV 2, Pregnancy, Hepatitis B, VZV IgG, VZV IgM, CMV, Toxoplasma, Lyme G/M and Hepatitis C. Immunoassay test systems manufactured for commercial distribution were used to determine the sero-positivity of the samples. Results for Hepatitis B and C and pregnancy testing were provided from alternate testing facilities. Ten samples for each possible cross-reactant were tested. The results presented were obtained by testing the analytes against high concentrations of possible cross reactants. The results of this study are summarized in the Table 13.

Table 13: Cross Reactivity

Analyte	Number Positive/ Number Tested
EBV	0/10
ANA	0/10
RF IgM	0/10
Rubella	0/10
HIV	0/10
HSV 1	0/10
HSV 2	0/10
Pregnancy	0/10
Hepatitis B	0/10
VZV	0/10
VZV IgM	0/10
CMV	0/10
Toxoplasma	0/10
Lyme G/M	0/10
Hepatitis C	0/10

4. Interfering Substances:

The effect of potential interfering substances on sample results generated using the assay was evaluated with the following possible interfering substances: albumin, bilirubin, cholesterol, hemoglobin, triglycerides and intralipids. The quantity of analyte in each interfering substance is as follows:

Bilirubin: 1mg/dL (low), 15 mg/dL (high)
Albumin: 3.5 g/dL (low), 5 g/dL (high)
Cholesterol: 150 mg/dL (low), 250 mg/dL (high)
Triglycerides: 150 mg/dL (low), 500 mg/dL (high)
Hemoglobin: 10 g/dL (low), 20 g/dL (high)
Intralipid: 300 mg/dL (low), 750 mg/dL (high)

All positive samples showed a change of signal less than 20%. The borderline samples showed a change of signal <20% of with the exception of the high spike of hemoglobin (25.2%). The negative sample showed a change of signal (>20%) with the high and low spikes

of albumin, hemoglobin, intralipid, bilirubin, cholesterol and triglycerides. The negative sample results in each instance stayed below the cut-off and the change in signal did not affect the qualitative result.

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GLOSSARY OF SYMBOLS

The following symbols **may** have been used in the labelling of this product or products associated with this product.

Symbol	Description	Symbol	Description
	Manufacturer		Keep away from sunlight
IVD	*In vitro* diagnostic medical device	PLATE	Plate
REF	Catalogue number	CONJ	Conjugate
Σ n	Sufficient for *n* tests	CTRL +	Positive Control
LOT	Batch code	CTRL -	Negative Control
	Use by	CAL	Calibrator
	Temperature limitation	DIL SPE	Sample Diluent
CONT	Contents	SOLN TMB	TMB
UDI	Unique Device Identifier	SOLN STOP	Stop Solution
	Consult the warnings and precautions	WASH 10X	Wash Buffer Concentrate (10X)
	Consult electronic instructions for use	EN	English
	Store in the upright position	Made in the USA	Made in the USA
RX Only	Applicable for U.S.A: Prescription *in vitro* diagnostic product		Corrosive
	Hazardous Communication	EC REP	European Commission Authorized Representative
CE	Conformity with Directive 98/79		

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