

INTENDED USE

The ZEUS AtheNA Multi-Lyte® *Treponema pallidum* IgG Plus Test System is a multiplex flow immunoassay intended for the qualitative detection of specific human IgG class antibodies to *Treponema pallidum* in human sera. The presence of antibodies to *T. pallidum* specific antigen, in conjunction with non treponemal laboratory tests and clinical findings, may aid in the diagnosis of syphilis infection. 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 *Borellia burgdorferi* and *Leptospira*. *Treponema 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 such techniques as dark field microscopy, direct fluorescent antibody stains, silver stains and serologic test (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 test. Serologic tests for syphilis include Non-treponemal test (VDRL) and Treponemal FTA or EIA (1, 3). Currently, the standard for syphilis testing is considered to be FTA-ABS and MHA-TP (2).

Both treponemal and non-treponemal 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).

PRINCIPLE OF THE ASSAY

The ZEUS AtheNA Multi-Lyte *T. pallidum* IgG Plus Test System is designed to detect IgG class antibodies in human sera to *T. pallidum*. The test procedure involves two incubation steps:

1. Test sera (properly diluted) are incubated in a vessel containing a multiplexed mixture Bead Suspension. The Bead Suspension contains a mixture of distinguishable sets of polystyrene microspheres (beads) each conjugated with *T. pallidum* antigen (p17kDa). If present in patient sera, specific antibodies will bind to the immobilized antigen on the bead set. The beads are rinsed to remove non-reactive serum proteins.
2. Phycoerythrin-conjugated goat anti-human IgG is added to the vessel and the plate is incubated. The Conjugate will react with IgG antibody immobilized on the solid phase in step 1. The Bead Suspension is then analyzed by the **AtheNA Multi-Lyte** instrument. The bead set(s) are sorted (identified) and the amount of reporter molecule (PE conjugate) is determined for each bead set. Using the *Intra-Well Calibration Technology*®, internal calibration bead sets are used to convert raw fluorescence into outcome (units).

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): Bead Suspension, Controls, Conjugate and SAVE Diluent®.**

SOLN	BEAD	1. Bead Suspension: Contains separate distinguishable 5.6 micron polystyrene beads that are conjugated with recombinant <i>T. pallidum</i> antigen. The Bead Suspension also contains one bead set designed to detect non-specific antibodies in the patient sample (if present) and four separate bead sets used for assay calibration. One, amber bottle containing 5.5mL. Ready to use.
CONJ		2. Conjugate: Phycoerythrin conjugated goat anti-human IgG (γ chain specific). One, amber bottle containing 15mL. Ready to use.
CONTROL	+	3. Positive Control (Human Serum): One, red-capped vial containing 0.2mL.
CONTROL	-	4. Negative Control (Human Serum): One, green-capped vial containing 0.2mL.
DIL	SPE	5. SAVE Diluent®: One, green-capped bottle containing 50mL of phosphate-buffered-saline. Ready to use. NOTE: The SAVE Diluent® will change color when combined with serum.
WASHBUF	10X	6. Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. One, clear-capped bottle containing containing 50mL of 10X concentrated phosphate-buffered-saline.

NOTES:

1. The following components are not Test System Lot Number dependent and may be used interchangeably with the ZEUS AtheNA Multi-Lyte Test Systems: Wash Buffer and SAVE Diluent®
2. Test System also contains:
 - a. Component Label containing lot specific information inside the Test System box.
 - b. Calibration CD containing lot specific kit calibration values required for specimen analysis and assay quality control, and Package Inserts.
 - c. One 96-well dilution plate.
 - d. One 96-well filter plate.

PRECAUTIONS

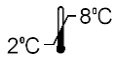
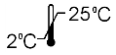
1. For *In Vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The **AtheNA Multi-Lyte** Bead Suspension does not contain viable organisms. However, the reagent should be considered **potentially biohazardous materials** and handled 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, handle these products 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 (4, 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. Do not allow the wells to dry out between incubations.

7. The SAVE Diluent®, Bead Suspension, Controls, and Conjugate 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 on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
8. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
9. Dilution or adulteration of these reagents may generate erroneous results.
10. Do not use reagents from other sources or manufacturers.
11. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
12. Avoid microbial contamination of reagents. Incorrect results may occur.
13. Cross contamination of reagents and/or samples could cause erroneous results.
14. Avoid splashing or generation of aerosols.
15. Do not expose reagents to strong light during storage or incubation. The Bead Suspension and Conjugate are light sensitive reagents. Both have been packaged in light protective containers. Normal exposures experienced during the course of performing the assay will not affect assay performance. Do not expose these reagents to strong sources of visible light unnecessarily.
16. 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.
17. Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
18. 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.
19. 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.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes capable of accurately delivering 10 - 200µL.
2. Multichannel pipette capable of accurately delivering 10 - 200µL.
3. Reagent reservoirs for multichannel pipettes.
4. Serological pipettes.
5. Disposable pipette tips.
6. Paper towels.
7. Laboratory timer to monitor incubation steps.
8. Disposal basin and disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite).
9. **AtheNA Multi-Lyte** System (Luminex® Instrument) with Sheath Fluid (Product Number 40-50000).
10. Distilled or deionized water.
11. Vortex.
12. Small Bath Sonicator.
13. Plate shaker capable of shaking at 800 RPM (optional for mixing).
14. Vacuum aspirator and vacuum manifold for washing the microspheres.

STORAGE CONDITIONS

	Bead Suspension: Remove only the required amount to analyze the specimens to be tested and return the unused portion to storage.
	Conjugate: DO NOT FREEZE. Unopened Test System, Positive Control, Negative Control, SAVE Diluent®
	Wash Buffer (1X): 20 - 25°C for up to 7 days, 2 - 8°C for 30 days.
	Wash Buffer (10X): 2 - 25°C

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. 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 (8).

ASSAY PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20 - 25°C).
2. Determine the total number of Controls and samples to be tested. It is necessary to include the Negative and Positive Control with each run. The Negative Control should be tested in well A1 and Positive Control in well B1. Each Control and sample requires one microwell for processing.
 - a. To optimize read times, the Bead Suspension must be thoroughly mixed just prior to use. The most effective for re-suspension is to first vortex for approximately 30 seconds followed by sonication for approximately 30 seconds in a small bath sonicator.
 - b. For proper performance, it is important that the contents of the assay are thoroughly mixed. Suitable means of mixing include mixing the plate on a plate shaker for approximately 30 seconds at approximately 800 RPMs or to set a pipettor to roughly ½ of the volume in the plate and repeatedly aspirate and expel (pump up and down) the contents of the well for a minimum of 5 cycles.

EXAMPLE PLATE SET-UP		
	1	2
A	Negative Control	Etc.
B	Positive Control	
C	Patient 1	
D	Patient 2	
E	Patient 3	
F	Patient 4	
G	Patient 5	
H	Patient 6	

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of SAVE Diluent*) of the Negative Control, 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.** For proper performance, it is important that the sample dilutions are thoroughly mixed according to 2b above.
4. After determining the total number of wells to process, use a multichannel or a repeating pipette to dispense 50µL of the Bead Suspension into each of the wells of the filtration plate.
5. Transfer 10µL of each diluted sample (1:21) and Control from the dilution plate to the filtration plate. For proper performance, it is important that the sample dilution and Bead Suspension are thoroughly mixed according to 2b above.
6. Incubate the plate at room temperature (20 - 25°C) for 30 ± 10 minutes.
7. After the incubation, rinse the Beads by vacuum filtration using the supplied Wash Buffer diluted to the 1X concentration.
 - a. Place the filtration plate on the vacuum manifold and remove the solution, leaving the beads behind.
 - b. Turn off the vacuum and add 200µL of 1X Wash Buffer.
 - c. Apply the vacuum and remove the solution.
 - d. Repeat steps 7b and 7c for a total of three rinses.
8. Following the final wash, gently blot the bottom of the filter plate and allow the plate to air dry for 3 - 5 minutes before proceeding to the next step.
9. Add 150µL of the Conjugate to each well, at the same rate and same order as the specimens. For proper performance, it is important that the Conjugate and Bead Suspension are thoroughly mixed according to 2b above. As an option, while mixing the Conjugate one may transfer the mixture to empty wells of a polystyrene reaction plate.
10. Incubate the plate at room temperature (20 - 25°C) for 30 ± 10 minutes.
11. Set the **AtheNA Multi-Lyte** instrument to analyze the reactions by selecting the *T. pallidum* IgG Plus template. Refer to the operators manual for details regarding the operation of the **AtheNA Multi-Lyte** instrument. Results may be read from the filter plate or a reaction plate. **NOTE: For proper specimen analysis, it is important that the instrument is set-up, calibrated and maintained according to the manufacturer's instructions.** Please review the instrument manual for instrument preparation prior to reading the assay results.
12. The plate should be read within 60 minutes after the completion of the Conjugate incubation. One may decide to shake the plate for approximately 15 seconds prior to reading. This optional step may reduce the amount of time required to read the plate.

Step	Abbreviated Assay Procedure
1	Dilute specimens 1:21 in SAVE Diluent*. Mix well.
2	Combine 50µL of Bead Suspension and 10µL of diluted specimen in an empty well. Mix well.
3	Incubate at room temperature for 30 ± 10 minutes.
4	Rinse the microspheres 3 times with 200µL of 1X Wash Buffer.
5	Gently blot the bottom of the plate and air dry for 3 - 5 minutes.
6	Add 150µL of Conjugate to each well. Mix well.
7	Transfer to a reaction plate (optional).
8	Incubate at room temperature for 30 ± 10 minutes
9	Shake plate (optional).
10	Read results within 60 minutes.

QUALITY CONTROL

- Caution:** The Negative and Positive Controls are intended to monitor for substantial reagent failure. The Positive Control will not ensure precision at the assay cutoff.
1. Each time the assay is run it is necessary to include the Negative Control (in well A1) and the Positive Control (in well B1).
 2. Run validity is determined through the performance of the Positive and Negative Controls. These criteria are analyzed automatically through *Intra-Well Calibration Technology*.
 - a. The Negative and Positive Controls must all be negative on the non-specific or control antigen bead.
 - b. The Negative Control must be negative for each and every analyte included in the Bead Suspension.
 - c. The Positive Control must be positive for the analytes for *T. pallidum*.
 - d. The ranges for the Positive Control and calibration calculations are lot specific and are encoded within the Calibration CD. Positive Control ranges may be viewed by clicking on the "Control Graphs" button of the **AtheNA Multi-Lyte** software and then clicking "Control Upper/Lower Limits".
 - e. If any of the above criteria are not met, the entire run will be considered invalid and should be repeated. **Do not report the patient results.**
 3. Specimen validity is based upon the characteristics of the calibration beads and their interactions with the patient sera. There are various parameters monitored automatically through *Intra-Well Calibration Technology*. If any of the criteria are found to be out of specification, the patient's results are considered invalid and should be repeated. Should this occur, the data report will indicate the particular specimen which has been invalidated as well as a trouble shooting code.
 4. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. External Controls must be representative of normal human serum since **AtheNA Multi-Lyte's** calibration system is partially based upon the characteristics of the serum sample. If the specimen formulation is artificial (not human serum), erroneous results may occur.
 5. Good laboratory practice recommends the use of positive and negative controls to assure functionality of reagents and proper performance of the assay procedure. Quality control requirements must be performed in conformance with local, state and/or federal regulations or accreditation requirements and the user's laboratory standard Quality Control procedures. It is recommended that the user refer to CLSI EP12-A and 42 CFR 493.1256 for guidance on appropriate QC practices (7).

INTERPRETATION OF RESULTS

1. **Calculations**
 - a. Assay Calibration: The ZEUS **AtheNA Multi-Lyte** *T. pallidum* IgG Plus Test System utilizes *Intra-Well Calibration Technology*. *Intra-Well Calibration Technology* includes a multi-point standard curve within the Bead Suspension. With *Intra-Well Calibration Technology*, each well of the assay is calibrated internally without any user intervention. The standard curve is designed to self-adjust based upon the unique characteristics of the patient or Control serum. Calibrator values are assigned to the internal standards by ZEUS, are lot specific and are encoded within the lot specific Calibration CD.
 - b. Analyte Cutoff Values: Each analyte of the ZEUS **AtheNA Multi-Lyte** *T. pallidum* IgG Plus Test System has an assigned cutoff value. Cutoff values are determined by ZEUS for each test system lot, and are encoded within the lot specific Calibration CD.
 - c. Through *Intra-Well Calibration Technology*, all calculations are performed automatically when using the **AtheNA Multi-Lyte** system. *Intra-Well Calibration Technology* performs a regression analysis of the internal standards and then adjusts the calculated unit values based upon an additional standard and the characteristics of the serum sample.
2. **Interpretations**
 - a. **Cutoff Determination:** The cut off for each assay was established using a negative population for each marker. The **AtheNA Multi-Lyte** results were determined for this population, and the cut off was set at approximately the mean plus three times the standard deviation. Based upon the results of this testing, the manufacturer has established the following guidelines for interpretation of patient samples.

b. **T. pallidum Analyte Interpretation**

Unit Value	Result	Interpretation
< 100 AU/mL	Negative	An AtheNA Multi-Lyte result of <100 AU/mL for the <i>T. pallidum</i> marker indicates no detectable IgG antibodies to that particular marker and should be reported as non-reactive for IgG antibody to <i>T. pallidum</i> .
100-120 AU/mL	Equivocal	Specimens with AtheNA Multi-Lyte results in the equivocal range (100 to 120 AU/mL) should be tested by an alternate serologic procedure, such as the ZEUS Scientific indirect fluorescent antibody (IFA) or other ELISA test procedures. Alternatively, a second freshly collected specimen sample should be collected and tested.
>120 AU/mL	Positive	An AtheNA Multi-Lyte result of >120 AU/mL for antibody to <i>T. pallidum</i> indicates that the specimen is positive for IgG antibody to <i>T. pallidum</i> , the causative agent for syphilis. A positive test result presumes a current or past infection with <i>T. pallidum</i> , and should be reported as reactive for IgG antibody to the marker. Other <i>T. pallidum</i> serology assays should be performed to confirm or rule out a current case of active syphilis.

c. **T. pallidum Result Interpretation**

Non-treponemal Result	Treponemal Result	Report/interpretation for all excluding neonates, infants and HIV-infected individuals*
Non-Reactive	Negative or Non-Reactive	No serologic evidence of infection with <i>T. pallidum</i> (incubating or early primary syphilis infection cannot be excluded).
Reactive	Negative or Non-Reactive	Current infection unlikely; probability of BFP secondary to other medical conditions (febrile diseases, immunizations, IVDU, autoimmune diseases, etc.). Recommend repeat testing (non-treponemal and treponemal by other test methods).
Non-Reactive	Positive or 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 or 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 or 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. The ZEUS **AtheNA Multi-Lyte T. pallidum** IgG Plus Test System is a diagnostic aid and by itself is not diagnostic. Test results should be interpreted 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. Do not perform testing as a screening procedure for the general population. The predictive value of a positive or negative result depends on the prevalence of analyte in a given patient population.
4. Hemolytic, icteric, or lipemic samples may interfere with the outcome of this assay. Additionally, specimens with abnormal IgG and RF antibody concentrations may interfere with the outcome of this assay. Avoid the use of these types of specimens.
5. Test results of specimens from immunosuppressed patients may be difficult to interpret.
6. Performance characteristics of this device have not been established for matrices other than serum.
7. 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.

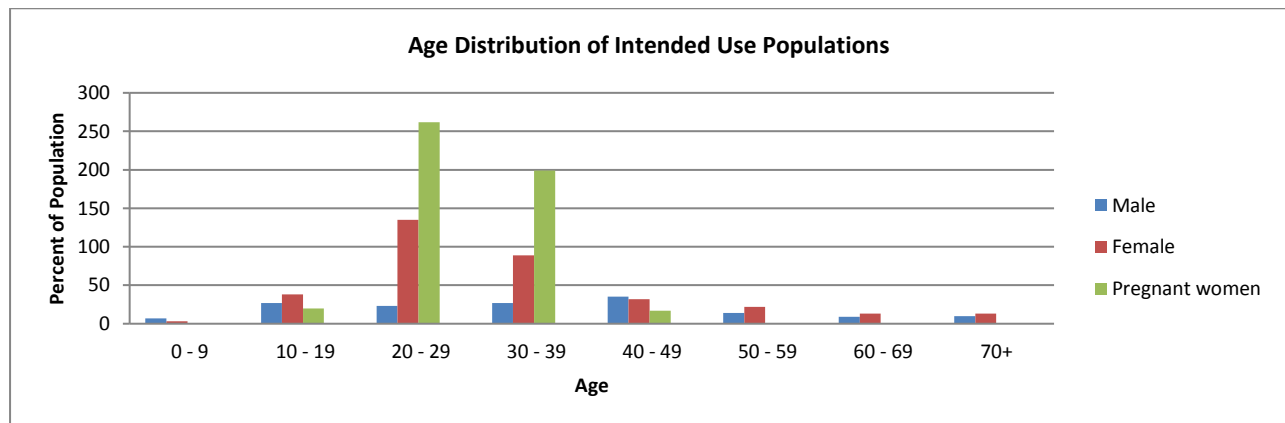
EXPECTED RESULTS

1. **Demographics And Age Distribution Of The Intended Use Populations**

Nine hundred and ninety-eight unselected samples, 500 each from individuals with a syphilis test requested and 498 from pregnant women with a syphilis test requested were tested. Site 1, 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 2, 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 3, 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 1 followed by the age distribution:

Table 1: 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	498	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 patients with a syphilis test ordered and 498 samples from pregnant women with a syphilis test ordered. 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. Site 1 was ZEUS Scientific. Site 2 was a hospital laboratory located in the northeast, and site 3 was a hospital laboratory located in the mid-Atlantic region.

Nineteen of 500 samples tested positive from the target population of patients with a syphilis test ordered ranging in age from <1 to >70 years old. The observed prevalence in this population was 3.8%. From this positive group of 19 individuals, 68.4% were females ranging in age from 30 to >70 (13/19, or 68.4%) and 31.6% were males ranging in age from 30 to >70 (6/19 or 31.6%). In the target group of pregnant women ranging in age from 17 to 49, 4/498 samples tested positive. The positive samples were from women in the 30 to 49 age group. The observed prevalence in this group of 498 pregnant women is 0.8%.

In the population of unselected hospitalized patients ranging in age from <1 to >70 years old, 50/ 1000 samples tested positive. The observed prevalence in this population was 5.0%. From the group of fifty positive specimens, 22/50 positive samples were from females ranging in age of 20 to >70 (44%) and 28/50 positive samples were from males ranging in age from <1 to >70 (56%). With respect to the data cited above, it should be noted that no additional studies or testing was done on any of the discrepant specimens in an attempt to determine the definitive serological status of these patient sera.

PERFORMANCE CHARACTERISTICS

1. **Comparative Studies**

A CDC panel of 164 characterized samples was tested. The summary of performance with the characterized serum panel appears in Table 2.

Table 2: Performance with CDC Characterized Serum Panel

Clinical Diagnosis	AtheNA Multi-Lyte® <i>T. pallidum</i> IgG Plus Results				% Agreement with Clinical Diagnosis Presented with 95% CI	
	Positive	Equivocal	Negative	Total		
Primary Treated	10	0	1	11	90.9	58.7 - 99.8
Secondary Untreated	40	0	3	43	93.0	80.8 - 98.5
Secondary Treated	39	0	0	39	100.0	92.6 - 100
Latent Untreated	6	0	5	11	54.5	23.4 - 83.3
Latent Treated	45	1	6	52	86.5	74.2 - 94.4
Congenital	2	0	1	3	66.7	9.4 - 99.2
Total	141	1	17	*159	88.7	82.7 - 93.2

*Only 159 of the original 164 specimens were available for testing by AtheNA Multi-Lyte®.

a. **Performance In Prospectively Collected Intended Use Populations**

The comparative study for the Intended Use Population consisted of 500 unselected serum samples from patients with a syphilis test ordered. Two hundred samples were tested at a hospital laboratory located in the Mid-Atlantic region. Two-hundred samples were tested at a hospital laboratory located in the Northeast and 100 samples were tested at ZEUS Scientific. Five-hundred purchased serum samples from pregnant women were tested: 100 at the manufacturer’s site, 200 at the hospital laboratory located in the Mid-Atlantic and 200 at the hospital laboratory located in the Northeast.

Table 3: Performance in a Population of Banked Sera from Individuals with a Syphilis Test Ordered

		Predicate					PPA/NPA	95% CI
		Positive	Equivocal	Negative	Site Total			
AtheNA Multi-Lyte <i>T. pallidum</i> IgG Plus	Positive	6		13	19	100.0%	60.7 - 100	
	Equivocal			4	4			
	Negative			477	477	96.6%	94.6 - 98	
	Invalid				0			
Site Total		6	0	494	500			

The summary of performance characteristics for 498 prospective samples collected from pregnant women follows. The samples were collected from pregnant women between the ages of 15 and 48 which had been submitted for Syphilis antibody testing.

Table 4: Performance in a Population of Banked Purchased Sera From Pregnant Women with a Syphilis Test Ordered

		Predicate					PPA/NPA	95% CI
		Positive	Equivocal	Negative	Site Total			
AtheNA Multi-Lyte <i>T. pallidum</i> IgG Plus	Positive	1		3	4	100.0%	50 - 100	
	Equivocal				0			
	Negative			494	494	99.4%	98.3 - 99.9	
	Invalid				0			
Site Total		1	0	497	498*			

*Note that 500 specimens were originally obtained; however, two serum specimens did not meet the inclusion criteria for the study.

Performance characteristics for 1000 unselected hospitalized patients may be viewed in table 5. These samples were pulled from the routine chemistry workload of a hospital laboratory.

Table 5: Performance in Unselected Hospitalized Patients

		Predicate					PPA/NPA	95% CI
		Positive	Equivocal	Negative	Site Total			
AtheNA Multi-Lyte <i>T. pallidum</i> IgG Plus	Positive	18		32	50	94.7%	74 - 99.9	
	Equivocal	1	1	8	10			
	Negative			932	932	95.9%	94 - 96.7	
	Invalid			4	4			
Site Total		19	1	976	996			

*Four of the original 1000 specimens were QNS for testing

b. **Performance In Retrospectively Collected Special Populations**

Comparative studies for the Special Populations were conducted at ZEUS Scientific. The retrospectively collected populations consisted of:

1. Table 6: 223 banked, known positive HIV-1 samples were purchased from a New York serum vendor.
2. Table 7: 277 banked samples from pregnant women were purchased from a New York serum vendor. 250 samples were RPR/TPPA negative and 27 were RPR/TPPA positive.
3. Table 8: 280 banked samples requested to be RPR/TPPA positive were purchased from a New York vendor.

Table 6: Performance in Banked Purchased Known HIV-1 Positive Individuals

		Predicate					
		Positive	Equivocal	Negative	Site Total	PPA/NPA	95% CI
AtheNA Multi-Lyte <i>T. pallidum</i> IgG Plus	Positive	46	3	5	54	97.9%	88.7 - 100
	Equivocal		2	2	4		
	Negative		1	164	165	94.3%	91.8 - 98.3
	Invalid				0		
Site Total		46	6	171	223		

Table 7: Performance in Banked Samples 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
AtheNA Multi-Lyte <i>T. pallidum</i> IgG Plus	Positive	26		9	35	96.3%	81 - 99.9
	Equivocal			1	1		
	Negative	1		240	241	96.0%	92.8 - 98.1
	Invalid						
Site Total		27	0	250	277		

Table 8: Performance in Banked Purchased Sera to be RPR/TPPA Positive Samples

		Predicate					
		Positive	Equivocal	Negative	Site Total	PPA/NPA	95% CI
AtheNA Multi-Lyte <i>T. pallidum</i> IgG Plus	Positive	275			275	99.6%	98.0 -100.9
	Equivocal	1			1		
	Negative			1	1	100%	50.0 -100
	Invalid				0		
Site Total		276	0	1	277*		

*Three of the original 300 specimens were QNS for testing.

c. Performance of AtheNA Multi-Lyte versus Panel of Sera obtained from the CDC

Three hundred and fifty-nine uncharacterized serum samples obtained from the CDC were evaluated.

Table 9: Performance of ZEUS AtheNA Multi-Lyte *T. pallidum* IgG Plus vs. Panel of Samples from the CDC

		TPPA					
		Positive	Equivocal	Negative	Site Total	PPA/NPA	95% CI
AtheNA Multi-Lyte <i>T. pallidum</i> IgG Plus	Positive	246	1	3	250	96.5%	93.4 - 98.2
	Equivocal			3	3		
	Negative	9		94	103	93.1%	86.2 - 97.2
	Invalid				0		
Site Total		255	1	100	356*		

*Three of the original 359 specimens to be included in this study were indeterminate by the CDC TPPA method and were excluded from the study.

2. Precision

Precision was evaluated internally at the manufacturer’s site. The study was conducted as follows: nine samples (negative, high negative, near cut-off, low positive and high positive) were identified and/or prepared (by ZEUS Scientific) for use in the study based upon their activity on the ZEUS **AtheNA Multi-Lyte® *T. pallidum* IgG Plus Test System**. 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. The test results for negative samples, less than 20 AU/mL is consistent with a high %CV which is acceptable. This was repeated for 20 days and the resulting data used to assess precision.

Table 10: ZEUS AtheNA Multi-Lyte *T. pallidum* IgG Plus Precision Study

Panel Member	Sample N	Mean AU/mL	Within-Run		Within -Day		Between-Run		Total	
			StD	%CV	StD	%CV	StD	%CV	StD	%CV
High Positive 1	80	352.68	19.2	5.4%	9.1	6.4%	8.8	2.5%	24.0	6.8%
High Positive 2	80	462.59	19.2	4.1%	11.1	4.3%	10.8	2.3%	26.8	5.8%
High Positive 3	80	481.24	24.8	5.2%	8.4	5.5%	12.4	2.6%	27.9	5.8%
Moderate Positive 1	80	210.11	16.2	7.4%	6.5	6.0%	12.3	5.5%	22.3	10.6%
Moderate Positive 2	80	317.40	20.2	6.3%	9.5	7.6%	11.0	3.4%	21.9	6.9%
Moderate Positive 3	80	248.46	16.4	6.5%	8.8	7.2%	8.8	3.5%	22.8	9.2%
Low Positive 1	80	181.63	9.3	5.1%	5.7	7.3%	4.9	2.7%	15.7	8.7%
Low Positive 2	80	144.20	6.7	4.6%	2.9	5.4%	4.3	3.0%	10.9	7.5%
Low Positive 3	80	164.10	8.7	5.4%	4.0	6.9%	8.1	5.1%	164.1	8.1%
Near Cut-off 1	80	111.25	4.0	3.7%	2.8	4.9%	2.1	1.9%	111.3	6.0%
Near Cut-off 2	80	108.94	6.0	5.3%	6.6	7.2%	4.8	4.3%	108.9	6.8%
Near Cut-off 3	80	109.91	4.0	3.7%	1.6	4.1%	3.0	2.7%	109.9	5.4%
High Negative 1	80	75.40	5.0	6.5%	3.1	9.2%	2.9	3.8%	75.4	10.5%
High Negative 2	80	84.80	6.6	7.7%	2.4	7.1%	3.1	3.7%	84.8	8.4%
High Negative 3	80	92.78	4.9	5.3%	3.1	6.0%	2.7	2.9%	92.8	6.8%
Negative 1	80	11.76	1.5	13.1%	0.7	18.0%	1.0	8.8%	11.8	18.8%
Negative 2	80	9.85	1.6	16.3%	1.0	16.5%	1.1	10.8%	9.9	20.4%
Negative 3	80	18.73	2.1	11.2%	1.3	15.7%	1.2	6.0%	18.7	17.0%
Non-Reactive Control	80	12.99	2.0	15.6%	2.25	17.6%	1.22	9.4%	2.48	19.08%
Reactive Control	80	903.76	38.87	4.3%	41.79	4.6%	23.85	2.6%	42.57	4.71%

3. Reproducibility

Reproducibility was evaluated internally and at two external clinical sites. The study was conducted as follows: Nine samples (negative, high negative, near cut-off, low positive and high positive) were identified and/or prepared (by ZEUS Scientific) for use in the study based upon their activity on the ZEUS **AtheNA Multi-Lyte® *T. pallidum* IgG Plus Test System**. 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 12 results per day. This was repeated for five days at each site and the resulting data used to assess reproducibility. The test results for negative samples, less than 20 AU/mL is consistent with a high %CV which is acceptable.

Table 11: Summary Of Multi-Site Reproducibility – ZEUS AtheNA Multi-Lyte *T. pallidum* IgG Plus

Panel Member	Sample N	Mean AU/mL	Within-Run		Within -Day		Between-Run		Between-Site		Total	
			StD	%CV	StD	%CV	StD	%CV	StD	%CV	StD	%CV
High Positive 1	180	326	19.2	5.8%	21.8	6.6%	12.7	3.8%	24.2	7.4%	26.6	8.2%
High Positive 2	180	436	21.9	5.0%	26.6	6.1%	19.2	4.4%	29.3	6.7%	32.6	7.5%
High Positive 3	180	459	23.8	5.2%	30.0	6.5%	21.2	4.6%	30.9	6.7%	33.1	7.2%
Mod Positive 1	180	196	10.6	5.4%	12.2	6.2%	7.0	3.6%	13.5	6.9%	14.6	7.5%
Mod Positive 2	180	304	16.3	5.3%	18.7	6.1%	11.2	3.7%	19.3	6.4%	21.2	7.0%
Mod Positive 3	180	234	14.0	5.9%	18.4	7.8%	13.9	5.9%	19.1	8.2%	21.1	9.0%
Low Positive 1	180	170	10.1	5.9%	12.1	7.1%	8.2	4.8%	13.0	7.7%	15.5	9.1%
Low Positive 2	180	137	7.8	5.7%	9.8	7.1%	6.9	4.9%	10.9	8.0%	11.7	8.5%
Low Positive 3	180	156	8.7	5.5%	11.2	7.1%	8.0	5.1%	12.3	7.9%	14.5	9.3%
Near Cut-off 1	180	106	6.6	6.1%	8.2	7.7%	6.1	5.7%	8.6	8.1%	9.8	9.2%
Near Cut-off 2	180	103	5.9	5.7%	7.2	6.9%	4.9	4.7%	7.5	7.3%	8.9	8.6%
Near Cut-off 3	180	108	6.3	5.8%	7.0	6.4%	3.3	3.1%	8.1	7.5%	9.4	8.8%
High Negative 1	180	73	4.6	6.3%	5.3	7.2%	2.8	3.8%	5.5	7.6%	6.5	8.8%
High Negative 2	180	81	5.6	6.9%	6.5	8.1%	3.9	4.8%	6.9	8.5%	7.4	9.2%
High Negative 3	180	88	5.2	5.8%	6.3	7.2%	4.1	4.6%	6.8	7.7%	7.6	8.7%
Negative 1	180	11	2.1	19.3%	2.2	20.1%	0.8	7.0%	2.2	20.1%	2.5	22.8%
Negative 2	180	9	1.8	20.2%	1.9	20.9%	0.5	6.0%	2.0	21.9%	2.2	24.9%
Negative 3	180	17	2.3	13.7%	2.4	14.0%	0.9	5.0%	2.4	13.8%	2.5	14.7%
Non-Reactive Control	90	12	2.3	18.7%	2.4	19.9%	1.2	9.7%	2.5	20.2%	2.8	23.3%
Reactive Control	90	886	32.2	3.6%	35.4	4.0%	18.3	2.1%	36.9	4.2%	38.9	4.4%

4. Cross Reactivity

Studies were performed at the manufacturing facility to assess cross reactivity with the ZEUS **AtheNA Multi-Lyte *T. pallidum* IgG Plus 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. Micro-particle and ELISA 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 minimally 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 following table.

Table 12: Cross Reactivity Summary

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
Elevated HCG	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

5. Interfering Substances

The effect of potential interfering substances on sample results generated using the ZEUS **AtheNA Multi-Lyte *T. pallidum* IgG Plus Test System** was evaluated with the following possible interfering substances based on the guidelines established in CLSI EP7-A2: 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: 20 g/dL (low), 20 g/dL (high)
- Intralipid: 300 mg/dL (low), 750 mg/dL (high)

Three samples each for *T. pallidum* IgG were chosen based on their performance on the ZEUS **AtheNA Multi-Lyte *T. pallidum* IgG Plus Test System**: positive, borderline and negative. The samples were exposed to the possible interfering substances and tested. All samples showed less than a 20% recovery of signal with the following exceptions:

- a. The positive sample showed a recovery of signal of 76% with the low spike of triglycerides, 60% with the high spike of hemoglobin and 74% with the high spike of intralipids.
- b. The borderline sample showed a recovery of signal of 74% with the low spike of albumin and 77% with the high spike of albumin. The high spikes of triglycerides and hemoglobin resulted in a recovery of signal of 76% for both substances.
- c. The negative sample showed a change of signal with several of the potential interfering substance at both the high and low spikes. The negative samples stayed below the cut-off of 100 AU/ml and the change in signal did not affect the qualitative result.

REFERENCES

1. Stevens RW and Schmitt ME: Evaluation of an Enzyme-linked Immunosorbent Assay for Treponemal Antibody. *Journal of Clinical Microbiology*. 21(3):399-402, 1985.
2. World Health Organization Technical Report Series: Treponemal Infections. No.674, 1982.
3. Farnes SW, and Setness P: Serologic Tests for Syphilis. *Post Graduate Medicine*. 87(3): 37-46, 1990.
4. U.S. Department of Health and Human Services. Public Health Service. Centers for Disease Control and Prevention and National Institutes of Health. U.S. Government Printing Office, Washington D.C., 4th Ed., 1999.
5. U.S. Department of Labor, Occupational Safety and Health Administration; Occupational Exposure to Bloodborne Pathogens, Final Rule. *Fed.Register* 56:64175-64182, 1991.
6. Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids and Tissues; Approved Guideline. CLSI Document M29, Vol.17(12), 1997.
7. CLSI. User Protocol for Evaluation of Qualitative Test Performance: Approved Guideline. CLSI document EP-12-A (ISBN 1-56238-468-6). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2002.
8. Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guidelines – 4th Edition (2010). CLSI Document GP44-A4 (ISBN 1-56238-724-3). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, PA 19087.



ZEUS Scientific, Inc.

200 Evans Way, Branchburg, New Jersey, 08876, USA

Toll Free (U.S.): 1-800-286-2111, Option 2

International: +1 908-526-3744

Fax: +1 908-526-2058

Website: www.zeusscientific.com

AtheNA Multi-Lyte and **SAVe Diluent**[®] are trademarks of ZEUS Scientific, Inc.

ZEUS **AtheNA Multi-Lyte** *T. pallidum* IgG Plus Test System

For US Customer Service contact your local distributor.

For US Technical Support contact ZEUS Scientific, call toll free

or e-mail support@zeusscientific.com.

For Non-US Customer Service and Technical Support inquiries, please contact your local distributor.

© 2017 ZEUS Scientific, Inc. All Rights Reserved.



(Rev. Date 12/18/2017)