HSV-1 & 2 IgG Plus Test System

REF A97101G

IVD

CE

Rx Only

INTENDED USE

The ZEUS **AtheNA Multi-Lyte®** Herpes Simplex Virus (HSV)-1 & 2 IgG Plus Test System is intended for the qualitative detection of presence or absence of IgG antibodies to HSV-1 and HSV-2 in human serum. The test is indicated for sexually active adults and expectant mothers, as an aid for presumptively diagnosing Herpes Simplex 1 and Herpes Simplex 2. The predictive value of positive or negative results depends on the population's prevalence and the pretest likelihood of HSV-1 and HSV-2. The test is not intended for donor screening or for self testing. The performance of this assay has not been established for use in a pediatric population, neonates, immunocompromised patients, for use by point of care facilities or for use with automated equipment. This test is for *In Vitro* diagnostic use only.

SIGNIFICANCE AND BACKGROUND

Herpes Simplex virus (HSV) infections are caused by two distinct antigenic types, HSV-1 and HSV-2 (1). Both HSV types are common human pathogens. HSV-1 is usually associated with infections in the oropharyngeal area and eyes while HSV-2 causes most genital and neonatal infections (1, 2). However, HSV-2 can be isolated occasionally from the oropharyngeal area (3) and 15 to 20% of primary genital infections may be caused by HSV-1 (1, 4).
HSV infections are transmitted by virus containing secretions through close personal contact. HSV infections, both primary and recurrent are often subclinical and asymptomatic. Shedding of the virus is the most important factor contributing to the spread of the virus (2).

Primary HSV-1 infections of the oral mucosa usually occur in children of less than 5 years of age (2). Most infections are asymptomatic. Symptomatic infections are characterized by gingivostomatitis associated with fever, malaise, and tender swollen cervical lymph nodes (2). Numerous small vesicles develop on the oral mucosa, become ulcerated, and heal within about two weeks. The most common form of recurrent HSV-1 is herpes labialis in which vesicles appear on the lips, nostrils or skin around the mouth (1, 2). Symptoms of genital HSV infections are multiple ulcerative lesions accompanied by pain, fever, dysuria, and lymphadenopathy (6).

The most severe complication of genital HSV infection is neonatal disease (2).Of mothers with an active primary infection, the risk of transmission to infants is as high as 40% (5). About 69 - 80% of infants who develop neonatal herpes are born to women who are asymptomatic of genital HSV infection at the time of birth (5). Genital herpes is problematic in sexually active adults as well as the disease is often transmitted in the absence of symptoms (13). HSV antibody testing is indicated for sexually active adults to identify those at risk for acquiring HSV or transmitting HSV to others and for expectant mothers who are at risk for acquiring HSV infections and transmitting neonatal herpes (7, 13).

Although culture combined with direct fluorescent antibody (DFA) testing is definitive in making a diagnosis, the timing is critical and cultures must be obtained during periods of active disease to produce optimal recovery (8, 9). Serological procedures may be useful for diagnosis of primary HSV infections, and for determining evidence of past infection with HSV (10). Many existing serologic methods for determining HSV sero-status, however, are unable to differentiate between HSV-1 and HSV-2 infections (10). Since the type of HSV implicated in disease has ramifications for prognosis (11, 12), it is important to specify the sub-type. HSV type-specific serological assays have been developed using the significant difference between the gG-1 protein of HSV 1 and the gG-2 protein of HSV 2 (10). Early application of type-specific serologic testing for HSV-1 and HSV-2 has been shown to benefit in testing first-time, recurrent, and asymptomatic infections as a means to definitive diagnosis and appropriate patient counseling (13). Serologic type-specific assays are useful in establishing or confirming the diagnosis of HSV-1 or 2 infections in asymptomatic people, those with symptomatic but negative culture lesions, and those with atypical presentations (14). Type-specific testing is recommended for sexually active adults and pregnant women as the presence of HSV antibodies is a reliable indicator that an individual may be infected with HSV and capable of transmitting the virus to others (14).

PRINCIPLE OF THE ASSAY

The ZEUS **AtheNA Multi-Lyte** HSV-1 & 2 IgG Plus Test System is designed to detect and differentiate IgG antibodies specific for HSV gG-1 and HSV gG-2 in human sera. 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) . Conjugated to the primary set of microspheres are HSV gG-1 and HSV gG-2 type-specific antigens. If present in patient sera, specific antibodies will bind to the immobilized antigen on one or more of the bead sets. 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 establish the assay's cutoff. Raw fluorescence from each distinct HSV gG-1 and HSV gG-2 antigen bead type is measured and compared against the cut-off calibrator.

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 HSV-1 recombinant gG-1 protein antigen, molecular weight 55 kDa and HSV-2 gG-2 recombinant protein antigen, molecular weight 31 kDa. 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 (15, 16).
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

2°C – 8°C	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®
2°C – 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

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 (21).

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 HSV-1 & 2 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

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*. The Negative and Positive controls are intended to monitor for substantial reagent failure. The Positive Control will not ensure precision at the assay cutoff.
   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. ThePositive Control must be positive for a predetermined group of analytes included in the Bead Suspension. These ranges are lot specific and are encoded within the Calibration CD. Postive 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".
   d. 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. If a specimen is repeatedly invalid, it must be tested using an alternate methodology since it is incompatible with the **AtheNA Multi-Lyte®** Plus Test System.
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 (27).

INTERPRETATION OF RESULTS

1. **Calculations**
   a. Assay Calibration: The ZEUS **AtheNA Multi-Lyte** HSV-1 & 2 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** HSV-1 & 2 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 this assay was originally optimized using a panel of negative specimens. Each subsequent kit lot has been tested against a panel of characterized specimens, and reported values are normalized using the lot specific Calibration CD.
   b. **HSV 1 and 2 Result Interpretation**: Specimen unit values for the analytes are interpreted as follows:

	AU/mL	Result	Interpretation
ZEUS **AtheNA Multi-Lyte** HSV-1 IgG Analyte	<100 AU/mL	Negative	No HSV-1 IgG antibodies were detected.
	>120 AU/mL	Positive	Presumptive for the prescence of HSV-1 IgG antibodies.
	100 – 120 AU/mL	Equivocal	Re-test the sample in duplicate. If on re-testing one of the two samples remain equivocal, the sample should be tested by an alternate type specific HSV serological procedure such as western blot, or a second sample should be drawn one to three weeks later.
	INV	Invalid	Indicates too much activity on the non-specific control bead. Re-test sample, if remains invalid, re-evaluate with a fresh sample.

	AU/mL	Result	Interpretation
ZEUS **AtheNA Multi-Lyte** HSV-2 IgG Analyte	<100 AU/mL	Negative	No HSV-2 IgG antibodies were detected.
	>120 AU/mL	Positive	Presumptive for the prescence of HSV-2 IgG antibodies.
	100 – 120 AU/mL	Equivocal	Re-test the sample in duplicate. If on re-testing one of the two samples remain equivocal, the sample should be tested by an alternate type specific HSV serological procedure such as western blot, or a second sample should be drawn one to three weeks later.
	INV	Invalid	Indicates too much activity on the non-specific control bead. Re-test sample, if remains invalid, re-evaluate with a fresh sample.

   1. The numeric value of the final result above the cut-off is not indicative of the amount of anti-HSV-1 and 2 IgG antibody present.
   2. Test results should be interpreted in conjunction with the clinical history, epidemiological data and other information available to the attending physician in evaluating the patient.
   3. False positive results may occur. Repeat testing or testing with a different device may be indicated in some settings, e.g. patients with a low likelihook of HSV infection.

LIMITATIONS OF THE ASSAY

1. The ZEUS **AtheNA Multi-Lyte** HSV-1 & 2 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. Hemolytic, icteric, or lipemic samples may interfere with the outcome of this assay. Additionally, specimens with abnormal IgG concentrations may interfere with the outcome of this assay. Use of these types of specimens should be avoided.
3. Performance characteristics of this device have not been established for matrices other than serum.
4. Samples collected too early in the course of the infection may not have detectable levels of HSV IgG. Negative HSV-2 results may be due to delayed seroconversion.
5. HSV serology cannot distinguish genital from non-genital infections.
6. Possible cross reactivity with *E.coli* , the recombinant vector for the gG1 and gG2 antigens, has not been evaluated.
7. The performance of this assay has been established using monoclonal antibodies for ruling out the following infectious agents which may produce symptoms similar to Herpes simplex: *Gonorrhoeae, Monobiluncus, Bacteroides, Gardnerella*, HPV and Chlamydia. The use of culture or other appropriate methods is recommended for these analytes.

EXPECTED RESULTS

1. Internal and external investigators assessed the device's performance in:
   a. Sexually active adults: 317 masked samples prospectively collected from sexually active adults between the ages of 17 and 69 which were submitted for HSV antibody testing. Site One, a hospital laboratory located in the northeast tested 135 samples collected in the western region of the United States. The samples were collected from 78 males and 48 females, 37 of which were of childbearing age. Nine samples were submitted with sex unknown. The median age of these patients was 35.4 years. Site Two, a hospital laboratory in the northeast tested 84 samples. The samples were collected from eight males and 76 females, 69 of which were woman of childbearing age. The median age of these patients was 48.7 years. Site Three, the manufacturer's facility, tested 98 samples collected in the northeast. The samples were collected from 46 males and 52 females of which 46 were of childbearing age. The median age of these patients was 36.7 years.
   b. Expectant mothers: 150 archived, masked samples were obtained from a serum vendor. The 150 expectant mothers ranged age from 18 to 48. Of these 150 expectant mothers, 50 were in their first trimester of pregnancy, 50 were in their second trimester and 50 were in their third trimester of pregnancy.
2. The observed prevalence and the hypothetical predictive values for the two populations are shown below. The calculations are based on the ZEUS **AtheNA Multi-Lyte** Test System having sensitivity and specificity of:
   a. HSV-1: sensitivity of 98.4% and specificity of 92.5% in sexually active adults and
   b. HSV-2: sensitivity of 97.6% and specificity of 96.2% in sexually active adults
   c. HSV-1: sensitivity of 100.0% and specificity of 96.2% in expectant mothers and
   d. HSV-2: sensitivity of 100.0% and specificity of 97.8% in expectant mothers.

   The observed prevalence of HSV-1 in the sexually active adult population is 59.6% and for HSV-2 it is 28.1%. In the expectant mother population, the observed prevalence for HSV-1 is 66.7% and for HSV-2 it is 40.7%.

Table 1: AtheNA Multi-Lyte HSV-1 & 2 IgG Plus Test System Expected Values/ Reference Ranges/Observed Prevalence (Sexually Active Adults)

Age	Sex	HSV-1 Positive	HSV-1 Negative	Total	Observed HSV-1 Prevelance (%)	HSV-2 Positive	HSV-2 Negative	Total	Observed HSV-2 Prevelance (%)
17 - 19	Male	4	1		2.1	0	5		0.0
	Female	7	8		3.7	2	12		2.2
20 - 29	Male	20	22		10.6	6	36		6.7
	Female	49	39		25.9	22	66		24.7
	Unknown	2	4		1.1	2	4		2.2
30 – 39	Male	16	18		8.5	8	26		9.0
	Female	31	7		16.4	12	27		13.5
	Unknown	1	2		0.5	1	2		1.1
40 – 49	Male	14	6		7.4	6	14		6.7
	Female	10	8		5.3	7	11		7.9
50 – 59	Male	19	5		10.1	12	12		13.5
	Female	6	3		3.2	3	6		3.4
	Unknown	1	0		0.5	1	0		1.1
60 - 69	Male	5	2		2.6	4	3		4.5
	Female	4	2		2.1	3	3		3.4
Sub-Total	Male	78	54		41.3	36	96		40.4
	Female	107	67		56.6	49	126		55.1
	Unknown	4	6		2.1	4	6		4.5
	Total	189	127	317	59.6	89	228	317	28.1

Table 2: ZEUS AtheNA Multi-Lyte HSV- 1 & 2 IgG Plus Test System Expected Values/ Reference Ranges/Observed Prevalence (Expectant Mothers)

Age	HSV-1 Positive	HSV-1 Negative	Total	Observed HSV-1 Prevelance (%)	HSV-2 Positive	HSV-2 Negative	Total	Observed HSV-2 Prevelance (%)
17 - 19	12	7		12.0	8	11		13.1
20 - 29	52	27		52.0	37	42		60.7
30 – 39	25	8		25.0	12	21		19.7
40 – 49	11	8		11.0	4	15		6.6
Total	100	50	150	66.7	61	89	150	40.7

Table 3: Prevalence vs. Hypothetical Predictive Value

Sexually Active Adults: Hypothetical Predicitive Values				
	HSV-1		HSV-2	
Prevalance	PPV	NPV	PPV	NPV
50%	92.3	98.3	96.2	97.6
40%	89.7	98.9	94.4	98.4
30%	84.9	99.3	91.6	98.9
25%	81.4	99.4	89.5	99.2
20%	76.	99.6	86.5	99.4
15%	69.8	99.7	81.9	99.6
10%	59.3	99.8	74.1	99.7
5%	40.8	99.9	57.5	99.9

Expectant Mothers: Hypothetical Predictive Values				
	HSV-1		HSV-2	
Prevalance	PPV	NPV	PPV	NPV
50%	96.3	100	97.8	100
40%	94.6	100	96.8	100
30%	91.9	100	95.1	100
25%	89.8	100	93.8	100
20%	86.8	100	91.9	100
15%	82.2	100	88.9	100
10%	74.5	100	83.5	100
5%	58.1	100	70.5	100

PERFORMANCE CHARACTERISTICS

1. **Comparative Study**

a. **Summary of Performance Characteristics**

		ZEUS **AtheNA Multi-Lyte** HSV-1 IgG	ZEUS **AtheNA Multi-Lyte** HSV-2 IgG
Sexually Active Adults (Intended Use Population)	Sensitivity (%)	98.4	97.6
	Specificity (%)	92.5	96.2
Expectant Mothers (Intended Use Population)	Sensitivity (%)	100.0	100.0
	Specificity (%)	96.2	97.8
CDC HSV Panel	Positives Agreement (%)	100.0	100.0
	Negatives Agreement (%)	100.0	98.1
Low Prevalence Population	Specificity (%)	96.7	98.4
Cross Reactivity	Cross Reactivity (%)	0.0	0.0
Reproducibility	% CV of Positives	<10.5	<15

b. **Performance in Sexually Active Adults**

ZEUS and two outside investigators assessed the device using a total of 317 prospective samples. ZEUS technicians tested 135 samples. Two outside investigators tested 84 samples and 98 samples respectively. The samples were sequentially submitted to the laboratories, archived and masked. The samples were collected from sexually active adults between the ages of 17 and 70 and submitted for Herpes simplex antibody testing. Results of this comparative study are individually presented by site and summarized in Tables 4 and 5.

Table 4: Sexually Active Adults (HSV-1)

			Predicate Immunoblot					
			Positive	Indeterminate	Negative	Site Total	Sensitivity/Specificity	95% CI
ZEUS **AtheNA Multi-Lyte** HSV-1 IgG	Site One	Positive	82	1	4	87	96.5 (82/85)	90.0 – 99.3
		Equivocal	0	0	0	0		
		Negative	3	0	45	48	91.8 (45/49)	80.4 – 97.7
		SiteTotal	85	1	49	135		
	Site Two	Positive	49	0	2	51	100.0 (49/49)	94.1 – 100.0
		Equivocal	0	1	0	1		
		Negative	0	0	32	32	91.4 (32/35)	77.0 – 98.2
		SiteTotal	49	0	35	84		
	Site Three	Positive	49	0	2	51	100.0 (49/49)	94.1 – 100.0
		Equivocal	0	0	0	0		
		Negative	0	0	47	47	95.9 (47/49)	86.0 – 99.5
		SiteTotal	49	0	49	98		
	Combined Sites	Positive	180	1	8	189	98.4 (180/183)	95.3 – 99.7
		Equivocal	0	0	1	1		
		Negative	3	0	124	127	92.5 (124/134)	86.7 – 96.3
		SiteTotal	183	1	133	317		

Table 5: Sexually Active Adults (HSV-2)

			Predicate Immunoblot					
			Positive	Indeterminate	Negative	Site Total	Sensitivity/Specificity	95% CI
ZEUS **AtheNA Multi-Lyte** HSV-2 IgG	Site One	Positive	43	0	2	45	97.7 (43/44)	88.0 – 99.9
		Equivocal	0	0	0	0		
		Negative	1	1	88	90	97.8 (88/90)	92.2 – 99.7
		SiteTotal	44	1	90	135		
	Site Two	Positive	16	0	3	19	100.0 (16/16)	82.9 – 100.0
		Equivocal	0	0	0	0		
		Negative	0	0	65	65	95.6 (65/68)	87.6 – 99.1
		SiteTotal	16	0	68	84		
	Site Three	Positive	21	0	4	25	100.0 (21/21)	86.7 – 100.0
		Equivocal	0	0	0	0		
		Negative	0	0	73	73	94.8 (73/77)	87.2 – 98.6
		SiteTotal	21	0	77	98		
	Combined Sites	Positive	80	0	9	89	97.6 (80/82)	91.4 – 99.7
		Equivocal	0	0	0	0		
		Negative	1	1	226	228	96.2 (226/235)	92.9 – 98.2
		SiteTotal	81	1	235	317		

c. **Performance in Expectant Mothers**

Comparative studies were performed at ZEUS using archived, masked sera obtained from a serum vendor. The 150 expectant mothers ranged from 18 to 48. Of these 150 expectant mothers, 50 were in their first trimester of pregnancy, 50 were in their second trimester and 50 were in their third trimester of pregnancy. The results appear in Tables 6 and 7.

Table 6: Expectant Mothers (HSV-1)

		Predicate Immunoblot Results			
		Positive	Indeterminate	Negative	Site Total
AtheNA Multi-Lyte	Positive	98	0	2	100
	Equivocal	0	0	0	0
	Negative	0	0	50	50
	Site Total	98	0	52	150
Sensitivity = 100.0% (98/98) *95% CI = 97.0 – 100.0					
Specificity = 96.2% (50/52) *95% CI = 86.8 – 99.5					

Table 7: Expectant Mothers (HSV-2)

		Predicate Immunoblot Results			
		Positive	Indeterminate	Negative	Site Total
AtheNA Multi-Lyte	Positive	59	0	2	61
	Equivocal	0	0	0	0
	Negative	0	0	89	89
	Site Total	59	0	91	150
Sensitivity = 100.0% (59/59) *95% CI = 95.1 – 100.0					
Specificity = 97.8% (89/91) *95% CI = 92.3 – 99.7					

*** Confidence Intervals calculated using the EXACT method.**

d. **Agreement with CDC Panel**

The performance of the ZEUS **AtheNA Multi-Lyte** HSV-1 & 2 IgG Plus Test System was assessed using a masked, well characterized HSV serum panel from the CDC. The panel consists of 24% HSV-1 and HSV-2 dual-positive samples, 50% HSV1 positive and 50% HSV-1 negative samples and 48% HSV-2 positive and 52% HSV2 negative samples. The results are presented to convey further information on the performance of the test kit and do not imply endorsement of the assay by the CDC. Results of this testing are presented in Tables 8 and 9.

Table8: CDC Panel (HSV-1)

		Positive	Negative	Site Total
AtheNA Multi-Lyte	Positive	50	0	50
	Equivocal	0	0	0
	Negative	0	50	50
	Site Total	50	50	100
Positive % Agreement = 100.0 (50/50) *95% CI = 94.2 – 100.0				
Negative % Agreement = 100.0 (50/50) *95% CI = 94.2 – 100.0				

Table 9: CDC Panel (HSV-2)

		Positive	Negative	Site Total
AtheNA Multi-Lyte	Positive	48	1	49
	Equivocal	0	0	0
	Negative	0	51	51
	Site Total	48	52	100
Positive % Agreement = 100.0 (48/48) *95% CI = 94.0 – 100.0				
Negative % Agreement = 98.1% (51/52) *95% CI = 89.7 – 100.0				

*** Confidence Intervals calculated using the EXACT method.**

e. **Performance in a Low Prevalence Population**

The relative specificity of the ZEUS **AtheNA Multi-Lyte** HSV-1 & 2 IgG Plus Test System was assessed internally using sera from a low prevalence population. The low prevalence population was comprised of sera stored in a serum bank at the manufacturer site. Archived, masked serum samples from 18 and 19 year old subjects previously tested for infections considered non-sexual in nature were tested and compared to the predicate device.

1. HSV-1 Reactivity: The predicate immunoblot device was positive for 8 samples and negative for 58 samples. The ZEUS **AtheNA Multi-Lyte** HSV-1& 2 IgG Plus Test System agreed with 100% (8/8) of immunoblot positives and 96.7% (56/58) of immunoblot negatives.
2. HSV-2 Reactivity: The predicate immunoblot device was positive for 3 samples and negative for 63 samples. The ZEUS **AtheNA Multi-Lyte** HSV-1& 2 IgG Plus Test System agreed with 100% (3/3) of immunoblot positives and 98.4% (62/63) of immunoblot negatives. The results of this study are summarized in Tables 10 and 11.

Table 10: Low Prevalence Population (HSV-1)

		Predicate Immunoblot Results			
		Positive	Indeterminate	Negative	Site Total
AtheNA Multi-Lyte	Positive	8	0	2	10
	Equivocal	0	0	0	0
	Negative	0	0	56	56
	Site Total	8	0	58	66
Sensitivity = 100.0% (8/8) *95% CI = 63.1 – 100.0					
Specificity = 96.7% (56/58) *95% CI = 88.1 – 99.6					

Table 11: Low Prevalence Population (HSV-2)

		Predicate Immunoblot Results			
		Positive	Indeterminate	Negative	Site Total
AtheNA Multi-Lyte	Positive	3	0	1	4
	Equivocal	0	0	0	0
	Negative	0	0	62	62
	Site Total	3	0	63	66
Sensitivity = 100.0% (3/3) *95% CI = 29.2 – 100.0					
Specificity = 98.4% (62/63) *95% CI = 91.2 – 100.0					

*** Confidence Intervals calculated using the EXACT method.**

1. **Precision and Reproducibility**

Assay reproducibility was evaluated internally and at two external clinical sites. For intra-assay reproducibility, the total %CV for HSV-1 is 9.7% and for HSV-2 it is 12.8%. The inter-assay reproducibility total %CV for HSV-1 is 10.5% and for HSV-2 it is 14.9%. The study was based on an internal SOP and conducted as follows: Six samples were identified and/or prepared (by Zeus Scientific, Inc.) for use in the study based upon their activity on the ZEUS **AtheNA Multi-Lyte** assay. Two samples were selected that were clearly negative, two that were clearly positive and two samples that were near the assay cut-off. To assess intra-assay precision, on each day of testing, each sample was diluted twice and then each dilution was run in quadruplicate, resulting in eight results per assay. This was repeated for three days and the resulting data used to assess inter-assay precision at each facility. To assess inter-laboratory precision, the results from each day's testing from each laboratory totaling nine runs, was used. The reproducibility studies are summarized in Tables 12 and 13.

Table 12: Reproducibility (HSV-1)

				Inter-Laboratory	
	Index Mean	Intra-Assay % CV	Inter-Assay %CV	Index Mean	Lab Mean % CV
Sample 1	26.3	15.0	18.2	26.3	21.8
Sample 2	8.4	39.2	44.0	8.4	58.2
Sample 3	144.8	11.9	15.9	144.8	16.6
Sample 4	195	11.0	12.3	195	15.9
Sample 5	311.8	8.4	9.9	311.8	10.7
Sample 6	392.2	8.5	9.1	392.2	12.8

Table 13: Reproducibility (HSV-2)

				Inter-Laboratory	
	Index Mean	Intra-Assay % CV	Inter-Assay %CV	Index Mean	Lab Mean % CV
Sample 1	16.4	36.4	36.8	16.4	44.0
Sample 2	20.8	27.9	31.0	20.8	40.0
Sample 3	155.7	115.5	21.0	155.7	23.6
Sample 4	114.2	10.6	13.7	114.2	18.1
Sample 5	44223	9.2	12.4	44223	13.9
Sample 6	356.2	8.0	14.1	356.2	16.1

2. **Repeatability**

Assay repeatability was evaluated at the manufacturer site in accordance with CLSI EP5: Evaluation of Precision Performance of Clinical Chemistry Devices-Second Edition,Wayne, PA (18). The study was conducted as follows: Six samples were identified and/or prepared (by ZEUS Scientific) for use in the study based upon their activity on the ZEUS **AtheNA Multi-Lyte** assay. Two samples were selected that were clearly negative, two that were clearly positive and two samples that were near the assay cut-off. On each day of testing, the samples were diluted twice and tested. This was repeated in a second run on the same day by a different technologist for a total of twelve days. This study is summarized in Table 14.

Table 14: Repeatability Results

	HSV-1			HSV-2		
	Index Mean	Intra-Assay % CV	Inter-Assay %CV	Index Mean	Intra-Assay % CV	Inter-Assay %CV
Sample 1	24.6	15.0	16.8	17.4	28.3	35.8
Sample 2	8.5	38.3	41.6	22.4	20.9	28.1
Sample 3	115.8	6.3	10.0	124	9.7	15.0
Sample 4	163.5	10.5	12.0	93.6	11.1	12.3
Sample 5	299.7	9.1	11.6	362	10.5	12.3
Sample 6	362.3	8.6	12.5	301.1	9.7	14.1

3. **Cross Reactivity**

Studies were performed to assess cross reactivity with the ZEUS **AtheNA Multi-Lyte** HSV-1 & 2 IgG Plus Test System using sera that were HSV dual-negative by immunoblot testing and that were sero-positive to Measles, Mumps, EBV VCA, EBNA, Rubella, VZV, ANA, CMV and Syphilis. ELISA and micro-particle immunoassay test systems manufactured by ZEUS Scientific for commercial distribution were used to determine the sero-positivity of the samples. Ten samples for each possible cross-reactant were tested. This study produced no detectable cross-reactivity with samples containing these various antibodies. Refer to Table 15 for the cross reactivity results.

Table 15: Cross Reactivity Results

Possible Cross-Reactants	Positive Results/Number Tested
Measles	0/10
Mumps	0/10
EBV-VCA	0/10
EBNA	0/10
Rubella	0/10
VZV	0/10
ANA	0/10
CMV	0/10
Syphilis	0/10

4. **Interfering Substances**

The effect of potential interfering substances on sample results generated using the ZEUS **AtheNA Multi-Lyte** HSV-1 & 2 IgG Plus Test System was evaluated with the following possible interfering substances based on the guidelines established in CLSI EP7-A2 (19): 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 HSV-1 and HSV-2 were chosen based on their performance on the ZEUS **AtheNA Multi-Lyte** Test System: positive (HSV-1, 818 AU/mL; HSV-2, 566 AU/mL), borderline (HSV-1, 152 AU/ml; HSV-2, 92 AU/mL) and negative (HSV-1, 62 AU/mL; HSV-2, 34AU/mL). The samples were exposed to the possible interfering substance, tested in duplicate and the mean established. All samples showed less than a 20% change in signal with the exception of the negative HSV-1 sample which exhibited an increase in signal of 33% with the low spike of albumin and an increase in signal of 39% with the high spike of albumin. The negative HSV-2 sample showed a change in signal of 37% with the low spike of albumin and 28% with the high spike of albumin. The negative HSV-2 sample also showed changes in signal with bilirubin, 43% and 52%, albumin, 37% and 28%, hemoglobin, 53% and 52% and intralipids, 52% and 34%, low and high spikes of interfering substances respectively. The change of signal in these negative samples did not change the qualitative outcome in these samples.

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