EBV IgG Plus Test System

REF **A92101G**

IVD

CE

Rx Only

INTENDED USE

The ZEUS **AtheNA Multi-Lyte®** Epstein-Barr Virus (EBV) IgG Plus Test System is intended for the qualitative detection of IgG class antibody to three separate EBV Antigens (EBV-VCA gp-125, total EBV-EA, and recombinant EBNA-1) in human serum using the AtheNA Multi-Lyte® System. The test system is intended to be used as an aid in the laboratory diagnosis of EBV-associated infectious mononucleosis and to provide epidemiological information on the disease caused by Epstein-Barr Virus. Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients, cord blood, neonatal specimens, or infants. Assay performance characteristics have not been established for the diagnosis of nasopharyngeal carcinoma, Burkitt's lymphoma, and other EBV-associated lymphomas. This test is for *In Vitro* diagnostic use only.

SIGNIFICANCE AND BACKGROUND

Epstein-Barr Virus is a ubiquitous human virus which causes infectious mononucleosis (IM), a self limiting lymphoproliferative disease (1). By adulthood virtually everyone has been infected and has developed immunity to the virus. In underdeveloped countries, seroconversion to the virus takes place in early childhood and is usually asymptomatic (2). In more affluent countries, primary EBV infections are often delayed until adolescence or later, and manifest as IM in about 50% of this age group (3 - 5).

Following seroconversion, whether symptomatic or not, EBV establishes a chronic, latent infection in B lymphocytes which probably lasts for life (6). EBV replicates in oropharyngeal epithelial cells and is present in the saliva of most patients with IM (7). Also, 10 - 20% of healthy persons who are EBV antibody positive shed the virus in their oral secretions (6, 7, 8). Reactivation of the latent viral carrier state, as evidenced by increased rates of virus shedding, is enhanced by immunosuppression, pregnancy, malnutrition, or disease (8, 9). Chronic EBV infections, whether latent or active, are rarely associated with disease.

The Paul-Bunnell-Davidsohn test for heterophile antibody is highly specific for IM (10). However, 10 - 15% of adults and higher percentages of children and infants with primary EBV infections do not develop heterophile antibodies (11). EBV-specific serological tests are needed to differentiate primary EBV infections that are heterophile negative from mononucleosis-like illnesses caused by other agents such as cytomegalovirus, adenovirus, and *Toxoplasma gondii* (4).

Antibody titers to specific EBV antigens correlate with different stages of IM (4, 10 - 12). Both IgM and IgG antibodies to the viral capsid antigen (VCA) peak three to four weeks after primary EBV infection. IgM anti-VCA decline rapidly and is usually undetectable after 12 weeks. IgG anti-VCA titers decline slowly after peaking but last indefinitely. Antibodies to EBV nuclear antigen (EBNA) develop from 1 month to 6 months after infection and, like anti-VCA, persist indefinitely (11, 12). Antibodies to EBNA indicate that the infection was not recent (11).

EBV early antigens (EA) consists of two components; diffuse (D), and restricted (R) (in this assay EA/D and EA/R are roughly equally distributed) . The terms D and R reflect the different patterns of immunofluorescence staining exhibited by the two components (13, 14). Antibodies to EA appear transiently for up to three months during the acute phase of IM in 85% of patients (15, 16). The antibody response to EA in IM patients is usually to the D component, whereas silent seroconversion to EBV in children produces antibodies to the R component (5, 11).

PRINCIPLE OF THE ASSAY

The ZEUS **AtheNA Multi-Lyte** EBV IgG Plus Test System is designed to detect IgG class antibodies in human sera to a variety of EBV antigens. 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); three of these bead sets are conjugated with the three EBV antigens (EBV-VCA, EBNA-1 and EBV-EA). 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 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®.**

Label		Component
SOLN	BEAD	1. Bead Suspension: Contains separate distinguishable 5.6 micron polystyrene beads that are conjugated with the following antigens: affinity purified EBV-VCA gp125, recombinant EA and recombinant EBNA-1. 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 (17, 18).
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 EBV 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*.
   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 all three analytes included in the Bead Suspension. These ranges are lot specific and are encoded within the Calibration CD. PC 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 (20).

INTERPRETATION OF RESULTS

1. **Calculations**
   a. Assay Calibration: The ZEUS **AtheNA Multi-Lyte** EBV 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** EBV 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. **EBV Analyte Interpretation:**

Unit Value	Result	Interpretation
<100 AU/mL	Negative	An **AtheNA Multi-Lyte** result of <100 AU/mL for any of the three EBV markers indicates no detectable IgG antibody to that particular marker and should be reported as non-reactive for IgG antibody to that marker. If all three markers are negative and exposure to Epstein-Barr virus is suspected, a second sample should be collected and tested no less than one to two weeks later.
100 - 120 AU/mL	Equivocal	Specimens with **AtheNA Multi-Lyte** results in the equivocal range (100 - 120 AU/mL) for any of the three markers should be tested by an alternate serologic procedure, such as the Zeus Scientific, Inc. 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 any of the three EBV markers indicates that the specimen is positive for IgG antibody to that marker. A positive test result presumes a current or past infection with EBV, and should be reported as reactive for IgG antibody to the marker(s). Other EBV serology assays such as the EBV VCA IgM should be performed to confirm serological status, active acute, past, or indeterminate infection for EBV-associated infectious mononucleosis.

c. Refer to the table below which shows the typical reactivity to the various EBV markers according to disease state (EBV seronegative, acute infection, past infection or indeterminate). If there is too much activity on the NSC (non-specific control) bead, *Intra-Well Calibration Technology* will invalidate that particular specimen. Specimens that are invalid should be re-tested. Specimens that are repeatedly invalid should be re-tested using an alternate procedure, such as the ZEUS IFA or ZEUS ELISA test procedures. The numeric value of the final result above the cutoff for any of the three EBV markers is not indicative of the amount of anti-EBV IgG antibody present.

EBV Classification	Heterophile	VCA IgM	VCA IgG	EBNA-1 IgG	EBV EA IgG[1]
Seronegative	Non-Reactive	Non-Reactive	Non-Reactive	Non-Reactive	Non-Reactive
	Not Available	Non-Reactive	Non-Reactive	Non-Reactive	Non-Reactive
Acute infection	Reactive	Reactive	Reactive	Non-Reactive	Reactive
	Reactive	Reactive	Non-Reactive	Non-Reactive	Reactive
	Non-Reactive	Reactive	Reactive	Non-Reactive	Reactive
	Reactive	Reactive	Reactive	Non-Reactive	Not Available
	Reactive	Reactive	Non-Reactive	Non-Reactive	Not Available
	Non-Reactive	Reactive	Reactive	Non-Reactive	Not Available
Past infection	Non-Reactive	Non-Reactive	Reactive	Reactive	Reactive
	Not Available	Non-Reactive	Reactive	Reactive	Reactive
	Non-Reactive	Non-Reactive	Reactive	Reactive	Not Available
	Not Available	Non-Reactive	Reactive	Reactive	Not Available
Indeterminate	Any combination not noted in the three categories above.				

[1] The EA antigen used for **AtheNA Multi-Lyte** contains roughly equal parts of EA/D and EA/R. Anti-EA/D shows a transient rise during acute infection, undetectable after 3 – 6 months. Anti-EA/R appears after EA/D and may be present greater than or equal to 2 years.

LIMITATIONS OF THE ASSAY

1. The ZEUS **AtheNA Multi-Lyte** EBV 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 with EBV-associated disease other than infectious mononucleosis.
4. Testing should not be performed 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. Testing should only be done when clinical evidence suggests the diagnosis of EBV-associated infectious mononucleosis.
5. Test results for anti-VCA should be interpreted in conjunction with the clinical evaluation and results of antibody tests for other EBV antigens, i.e., EBNA, EA, and IgG-VCA.
6. Test results of specimens from immunosuppressed patients may be difficult to interpret.
7. Performance characteristics of this device have not been established for matrices other than serum.
8. 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

The clinical study for the product included a total of 693 prospectively collected specimens and 70 retrospectively collected specimens for a total of 763 specimens. Aside from the samples tested at ZEUS, specimens were tested at three other facilities; a university medical center located in Eastern U.S. and two hospitals located in Northeastern United States. Of the 693 prospective specimens tested, 412 (tested at ZEUS and the university medical center) included demographics of the patient specimens (the two hospital sites did not include demographics with their test results), listed in Table 1. The histogram (Figure 1) depicts the age distribution of all 412 specimens while the other tables provide sexual distribution of the specimens. Table 2 shows a number of statistical results for the female and male patient specimens for each of the three assays.

Table 1: Patient Specimen Demographics

	Number of Samples	Mean	Median	Minimum	Maximum
Female Specimens	243	34.8	32.0	1	84
Male Specimens	169	35.8	33.0	1	83

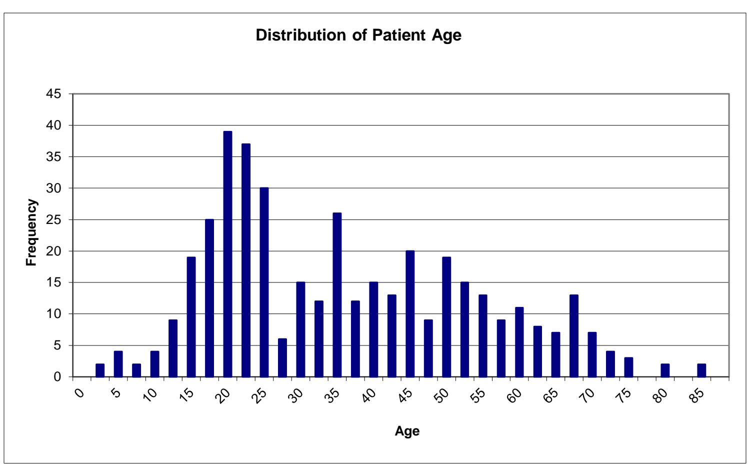

Figure 1: Age Distribution

Table 2: Statistical Results per Assay

	Female Specimens			Male Specimens		
	VCA G	EBNA	EA	VCA G	EBNA	EA
Mean (AU/mL)	352.6	626.5	150	353.5	611.6	145.3
Median (AU/mL)	365	747	116	324.5	729	115.5
Minimum (AU/mL)	17	7	17	22	7	13
Maximum (AU/mL)	847	1050	632	830	1065	604

EBV-VCA IgG Outcome:
For the female group, 81.1% (167/206) were positive, 18.0% (37/206) were negative, 1.0% (2/206) were equivocal and 0% (0/206) yielded invalid results. For the male group, 78.7% (107/136) were positive, 19.9% (27/136) were negative, 1.5% (2/136) were equivocal and 0% (0/136) yielded invalid results. With respect to the entire population of 763 specimens tested, 606/763 (79.4%) were positive, 148/763 (19.4%) were negative, 8/763 (1.0%) were equivocal and 1/763 (0.1%) were invalid.

EBNA IgG Outcome:
For the female group, 86.4% (178/206) were positive, 12.1% (25/206) were negative, 1.5% (3/206) were equivocal and 0% (0/206) yielded invalid results. For the male group, 86.8% (118/136) were positive, 13.2% (18/136) were negative, 0% (0/136) were equivocal and 0% (0/136) yielded invalid results. With respect to the entire population of 763 specimens tested, 547/763 (71.7%) were positive, 211/763 (27.7%) were negative, 4/763 (0.5%) were equivocal and 1/763 (0.1%) were invalid.

EBV-EA IgG Outcome:
For the female group, 49.5% (102/206) were positive, 46.6% (96/206) were negative, 3.9% (8/206) were equivocal and 0% (0/206) yielded invalid results. For the male group, 49.3% (67/136) were positive, 45.6% (62/136) were negative, 5.1% (7/136) were equivocal and 0% (0/136) yielded invalid results. With respect to the entire population of 763 specimens tested, 283/763 (37.1%) were positive, 452/763 (59.2%) were negative, 27/763 (3.5%) were equivocal and 1/763 (0.1%) were invalid.

PERFORMANCE CHARACTERISTICS

1. **Comparative Study**
   A multi-site comparative study was performed to evaluate the performance of the ZEUS **AtheNA Multi-Lyte** EBV IgG Plus Test System to the disease classification of the specimens as determined by other EBV serological reagents. Specimens were tested by reference ELISA assays for EBV-VCA IgG, EBNA IgG, EBV-VCA IgM and for heterophile antibody using a latex agglutination assay for purposes of classification into disease states. ELISA EBV-EA IgG results were not considered for purposes of classification of specimens into disease states. There were a total of 763 specimens tested. Of the 763 specimens tested, 693 were prospective specimens and 70 were retrospective specimens. The retrospective group was selected since clinical information suggested that they were representative of acute cases of infectious mononucleosis.Based upon the results of the three reference ELISA tests and the heterophile test, the specimens were categorized into one of 4 groups as indicated in Table 3.

Table 3: Specimen Population After Classification by Disease State

EBV Classification	Prospective Specimens	Retrospective Specimens	Heterophile	VCA IgG	VCA IgM	EBNA-1 IgG
Acute Infection	28	50	+	+	+	–
			+	–	+	–
			–	+	+	–
No Infection	95	1	–	–	–	–
			N/A	–	–	–
Past Infection	480	3	–	+	–	+
			N/A	+	–	+
Indeterminate	90	16	+	+	+	+
			+	+	–	+
			–	–	+	+
			–	+	+	+
			–	+	–	–
			–	–	+	–
			–	–	–	+
			N/A	+	–	–
Total:	693	70	+ = Reactive	– = Nonreactive	N/A = Not Available	

NOTE: When a reference assay result was equivocal, it was considered nonreactive (-).

Comparison data from the prospective acute, no infection, past infection and indeterminate population specimens (n = 693) appears in Tables 4 – 6.

Table 4: ZEUS AtheNA Multi-Lyte EBV-VCA IgG Plus Assay versus Comparative EBV-VCA IgG ELISA Assay

ELISA	Negative				Equivocal				Positive				Total N
AtheNA	Reactive	Nonreactive	Equivocal[1]	Invalid	Reactive	Nonreactive	Equivocal[1]	Invalid	Reactive	Nonreactive	Equivocal[1]	Invalid	
Acute	9	1	0	0	1	0	0	0	16	0	0	1	28
No Infection	2	92	1	0	0	0	0	0	0	0	0	0	95
Past Infection	0	0	0	0	0	0	0	0	441	35	4	0	480
Indeterminate	15	12	0	0	0	0	0	0	57	5	1	0	90
Overall	26	105	1	0	1	0	0	0	514	40	5	1	693

[1] Equivocal results following repeat testing.

Table 5: AtheNA Multi-Lyte EBNA IgG Plus Assay versus Comparative EBNA IgG ELISA Assay

ELISA	Negative				Equivocal				Positive				Total N
AtheNA	Reactive	Nonreactive	Equivocal[1]	Invalid	Reactive	Nonreactive	Equivocal[1]	Invalid	Reactive	Nonreactive	Equivocal[1]	Invalid	
Acute	4	23	0	1	0	0	0	0	0	0	0	0	28
No Infection	4	90	1	0	0	0	0	0	0	0	0	0	95
Past Infection	0	0	0	0	0	0	0	0	472	6	2	0	480
Indeterminate	20	24	1	0	0	0	0	0	41	4	0	0	90
Overall	28	137	2	1	0	0	0	0	513	10	2	0	693

[1] Equivocal results following repeat testing.

Table 6: ZEUS AtheNA Multi-Lyte EBV-EA IgG Plus Assay versus Comparative EBV-EA IgG ELISA Assay

ELISA	Negative				Equivocal				Positive				Total N
AtheNA	Reactive	Nonreactive	Equivocal[1]	Invalid	Reactive	Nonreactive	Equivocal[1]	Invalid	Reactive	Nonreactive	Equivocal[1]	Invalid	
Acute	3	19	0	1	0	1	0	0	1	3	0	0	28
No Infection	2	93	0	0	0	0	0	0	0	0	0	0	95
Past Infection	137	213	24	0	8	0	0	0	95	3	0	0	480
Indeterminate	15	59	1	0	0	0	0	0	13	2	0	0	90
Overall	157	384	25	1	8	1	0	0	109	8	0	0	693

[1] Equivocal results following repeat testing.

Comparison data from the retrospective expected acute, no infection, past infection and indeterminate population specimens (n = 70) appears inTables 7 - 9.

Table 7: ZEUS AtheNA Multi-Lyte EBV-VCA IgG Plus Assay versus Comparative EBV-VCA IgG ELISA Assay

ELISA	Negative				Equivocal				Positive				Total N
AtheNA	Reactive	Nonreactive	Equivocal[1]	Invalid	Reactive	Nonreactive	Equivocal[1]	Invalid	Reactive	Nonreactive	Equivocal[1]	Invalid	
Acute	12	1	1	0	1	1	0	0	34	0	0	0	50
No Infection	1	0	0	0	0	0	0	0	0	0	0	0	1
Past Infection	0	0	0	0	0	0	0	0	3	0	0	0	3
Indeterminate	7	1	0	0	1	0	0	0	6	0	1	0	16
Overall	20	2	1	0	2	1	0	0	43	0	1	0	70

[1] Equivocal results following repeat testing.

Table 8: ZEUS AtheNA Multi-Lyte EBNA IgG Plus Assay versus Comparative EBNA IgG ELISA Assay

ELISA	Negative				Equivocal				Positive				Total N
AtheNA	Reactive	Nonreactive	Equivocal[1]	Invalid	Reactive	Nonreactive	Equivocal[1]	Invalid	Reactive	Nonreactive	Equivocal[1]	Invalid	
Acute	2	48	0	0	0	0	0	0	0	0	0	0	50
No Infection	0	1	0	0	0	0	0	0	0	0	0	0	1
Past Infection	0	0	0	0	0	0	0	0	3	0	0	0	3
Indeterminate	0	4	0	0	0	0	0	0	1	11	0	0	16
Overall	2	53	0	0	0	0	0	0	4	11	0	0	70

[1] Equivocal results following repeat testing.

Table 9: ZEUS AtheNA Multi-Lyte EBV-EA IgG Plus Assay versus Comparative EBV-EA IgG ELISA Assay

ELISA	Negative				Equivocal				Positive				Total N
AtheNA	Reactive	Nonreactive	Equivocal[1]	Invalid	Reactive	Nonreactive	Equivocal[1]	Invalid	Reactive	Nonreactive	Equivocal[1]	Invalid	
Acute	1	25	0	0	1	3	1	0	4	14	1	0	50
No Infection	0	1	0	0	0	0	0	0	0	0	0	0	1
Past Infection	1	1	0	0	0	0	0	0	1	0	0	0	3
Indeterminate	0	10	0	0	0	0	0	0	1	5	0	0	16
Overall	2	37	0	0	1	3	1	0	6	19	1	0	70

[1] Equivocal results following repeat testing.

For purposes of percent agreement calculations, the ZEUS **AtheNA Multi-Lyte** EBV IgG Plus equivocal results were assigned to the opposite clinical interpretation than that of the comparative assay result. Likewise, the comparative assay equivocal results were assigned to the opposite clinical interpretation than that of the ZEUS **AtheNA Multi-Lyte** EBV IgG Plus result. The percent agreement between the ZEUS **AtheNA Multi-Lyte** EBV IgG Plus assays and the comparative EBV IgG ELISA assays are summarized in Tables 10 – 15.

Table 10: ZEUS AtheNA Multi-Lyte EBV-VCA IgG Plus Assay versus EBV-VCA IgG Reference ELISA Assay - Prospective Specimens

EBV Classification	Negative % Agreement (x/n)[b]	95% Exact Confidence Interval	Positive % Agreement (x/n)[a]	95% Exact Confidence Interval
Acute	9.1 (1/11)	0 – 26.1	94.1 (16/17)	82.9 – 100
No infection	97.9 (92/94)	95.0 - 100	N/A[c]	N/A
Past infection	N/A	N/A	91.9 (441/480)	89.4 - 94.3
Indeterminate	44.4 (12/27)	25.7 - 63.2	90.5 (57/63)	83.2 - 97.7
Overall	78.9 (105/133)	72.0 - 85.9	91.8 (514/560)	89.5 - 94.1

a x = the number of ZEUS **AtheNA Multi-Lyte** EBV-VCA IgG Plus results that are confirmed positive in agreement with the reference EBV-VCA IgG confirmed positive results; n = the total number of reference EBV-VCA IgG results that are confirmed positive.

b x = the number of ZEUS **AtheNA Multi-Lyte** EBV-VCA IgG Plus results that are nonreactive in agreement with the reference EBV-VCA IgG; n = the total number of reference EBV-VCA IgG results that are nonreactive.

c Agreement resulted in 0/0 specimens. In such cases, percent agreement and 95% confidence intervals could not be calculated.

Table 11: ZEUS AtheNA Multi-Lyte EBNA IgG Plus Assay versus EBNA IgG Reference ELISA Assay - Prospective Specimens

EBV Classification	Negative % Agreement (x/n)[b]	95% Exact Confidence Interval	Positive % Agreement (x/n)[a]	95% Exact Confidence Interval
Acute	85.2 (23/27)	71.8 – 98.6	N/A[c]	N/A
No infection	95.7 (90/94)	91.7 - 99.8	N/A	N/A
Past infection	N/A	N/A	98.3 (472/480)	97.2 - 99.5
Indeterminate	53.3 (24/45)	38.8 - 67.9	91.1 (41/45)	82.8 - 99.4
Overall	77.0 (137/178)	70.8 - 83.2	97.7 (513/525)	96.4 - 99.0

a x = the number of ZEUS **AtheNA Multi-Lyte** EBNA IgG Plus results that are confirmed positive in agreement with the reference EBNA IgG confirmed positive results; n = the total number of reference EBNA IgG results that are confirmed positive.

b x = the number of ZEUS **AtheNA Multi-Lyte** EBNA IgG Plus results that are nonreactive in agreement with the reference EBNA IgG; n = the total number of reference EBNA IgG results that are nonreactive.

c Agreement resulted in 0/0 specimens. In such cases, percent agreement and 95% confidence intervals could not be calculated.

Table 12: ZEUS AtheNA Multi-Lyte EBV-EA IgG Plus Assay versus EBV-EA IgG Reference ELISA Assay - Prospective Specimens

EBV Classification	Negative % Agreement (x/n)[b]	95% Exact Confidence Interval	Positive % Agreement (x/n)[a]	95% Exact Confidence Interval
Acute	79.2 (19/24)	62.9 – 95.4	25.0 (1/4)	0 – 67.4
No infection	97.9 (93/95)	95.0 – 100	N/A[c]	N/A
Past infection	55.8 (213/382)	50.8 - 60.7	96.9 (95/98)	93.5 – 100
Indeterminate	78.7 (59/75)	69.4 - 87.9	86.7 (13/15)	69.5 – 100
Overall	66.7 (384/576)	62.8 - 70.5	93.2 (109/117)	88.6 - 97.7

a x = the number of ZEUS **AtheNA Multi-Lyte** EBV-EA IgG Plus results that are confirmed positive in agreement with the reference EBV-EA IgG confirmed positive results; n = the total number of reference EBV-EA IgG results that are confirmed positive.

b x = the number of ZEUS **AtheNA Multi-Lyte** EBV-EA IgG Plus results that are nonreactive in agreement with the reference EBV-EA IgG; n = the total number of reference EBV-EA IgG results that are nonreactive.

c Agreement resulted in 0/0 specimens. In such cases, percent agreement and 95% confidence intervals could not be calculated.

Table 13: ZEUS AtheNA Multi-Lyte EBV-VCA IgG Plus Assay versus EBV-VCA IgG Reference ELISA Assay - Retrospective Specimens (Expected Acute)

EBV Classification	Negative % Agreement (x/n)[b]	95% Exact Confidence Interval	Positive % Agreement (x/n)[a]	95% Exact Confidence Interval
Acute	6.3 (1/16)	0 – 18.1	100 (34/34)	100 – 100
No infection	0.0 (0/1)	0 – 0	N/A[c]	N/A
Past infection	N/A	N/A	100 (3/3)	100 – 100
Indeterminate	11.1 (1/9)	0 - 31.6	85.7 (6/7)	59.8 – 100
Overall	7.7 (2/26)	0 - 17.9	97.7 (43/44)	93.3 – 100

a x = the number of ZEUS **AtheNA Multi-Lyte** EBV-VCA IgG Plus results that are confirmed positive in agreement with the reference EBV-VCA IgG confirmed positive results; n = the total number of reference EBV-VCA IgG results that are confirmed positive.

b x = the number of ZEUS **AtheNA Multi-Lyte** EBV-VCA IgG Plus results that are nonreactive in agreement with the reference EBV-VCA IgG; n = the total number of reference EBV-VCA IgG results that are nonreactive.

c Agreement resulted in 0/0 specimens. In such cases, percent agreement and 95% confidence intervals could not be calculated.

Table 14: ZEUS AtheNA Multi-Lyte EBNA IgG Plus Assay vs. EBNA IgG Reference ELISA Assay - Retrospective Specimens (Expected Acute)

EBV Classification	Negative % Agreement (x/n)[b]	95% Exact Confidence Interval	Positive % Agreement (x/n)[a]	95% Exact Confidence Interval
Acute	96.0 (48/50)	90.6 – 100	N/A[c]	N/A
No infection	100 (1/1)	100 - 100	N/A	N/A
Past infection	N/A	N/A	100 (3/3)	100 – 100
Indeterminate	100 (4/4)	100 - 100	8.3 (1/12)	0 - 24.0
Overall	96.4 (53/55)	91.4 - 100	26.7 (4/15)	4.3 - 49.0

a x = the number of ZEUS **AtheNA Multi-Lyte** EBNA IgG Plus results that are confirmed positive in agreement with the reference EBNA IgG confirmed positive results; n = the total number of reference EBNA IgG results that are confirmed positive.

b x = the number of ZEUS **AtheNA Multi-Lyte** EBNA IgG Plus results that are nonreactive in agreement with the reference EBNA IgG; n = the total number of reference EBNA IgG results that are nonreactive.

c Agreement resulted in 0/0 specimens. In such cases, percent agreement and 95% confidence intervals could not be calculated.

Table 15: ZEUS AtheNA Multi-Lyte EBV-EA IgG Plus Assay vs. EBV-EA IgG Reference ELISA Assay - Retrospective Specimens (Expected Acute)

EBV Classification	Negative % Agreement (x/n)[b]	95% Exact Confidence Interval	Positive % Agreement (x/n)[a]	95% Exact Confidence Interval
Acute	80.6%(25/31)	66.7% – 94.6%	21.1%(4/19)	2.7% – 39.4%
No infection	100%(1/1)	100% - 100%	N/A[c]	N/A
Past infection	50.0%(1/2)	0% - 100%	100%(1/1)	100% - 100%
Indeterminate	100%(10/10)	100% - 100%	16.7%(1/6)	0% - 46.5%
Overall	84.1%(37/44)	73.3% - 94.9%	23.1%(6/26)	6.9% - 39.3%

a x = the number of ZEUS **AtheNA Multi-Lyte** EBV-EA IgG Plus results that are confirmed positive in agreement with the reference EBV-EA IgG confirmed positive results; n = the total number of reference EBV-EA IgG results that are confirmed positive.

b x = the number of ZEUS **AtheNA Multi-Lyte** EBV-EA IgG Plus results that are nonreactive in agreement with the reference EBV-EA IgG; n = the total number of reference EBV-EA IgG results that are nonreactive.

c Agreement resulted in 0/0 specimens. In such cases, percent agreement and 95% confidence intervals could not be calculated.

2. **Precision**

Precision was evaluated at all three clinical sites. To evaluate both intra-assay and inter-assay reproducibility, six specimens were tested. On each day of testing, each sample was diluted twice and then loaded for four replicates resulting in a total of eight wells of each of the six samples. This protocol was followed for three days. These results were then used to calculate mean U/mL values, standard deviations, and percent CV. At each site, specimens were selected in such a way that resulted in some of them being clearly negative, some being clearly positive and some were selected that were weakly positive or just near the cutoff of the assay. A summary of this testing appears in Table 16.

Table 16: Precision Testing

			EBV-VCA						EBNA						EBV-EA					
			Panel 1	Panel 2	Panel 3	Panel 4	Panel 5	Panel 6	Panel 7	Panel 8	Panel 9	Panel 10	Panel 11	Panel 12	Panel 13	Panel 14	Panel 15	Panel 16	Panel 17	Panel 18
Site 1		Mean	603	481	80	96	43	44	941	1041	110	43	32	23	785	433	100	141	78	61
	Within Day 1	StD	20.12	16.88	13.15	11.09	3.62	4.62	55.26	39.14	8.85	5.42	2.38	2.93	48.68	23.55	6.65	6.25	5.62	7.78
		%CV	3.4	3.5	14.6	10.7	8.0	10.4	6.0	3.8	7.9	11.4	7.1	11.9	6.3	5.4	6.5	4.4	6.8	12.0
	Within Day 2	StD	28.17	27.84	4.77	7.61	2.83	4.14	48.13	30.75	18.41	3.65	6.00	2.72	47.35	37.22	3.82	14.79	6.95	8.94
		%CV	4.7	5.9	5.9	8.3	6.8	9.3	5.2	2.9	16.1	9.6	9.3	11.6	6.0	8.9	3.9	10.1	9.1	14.6
	Within Day 3	StD	50.64	44.12	5.96	7.44	3.29	5.68	53.18	76.73	7.38	5.15	2.38	2.45	86.79	38.29	11.97	8.68	11.20	12.08
		%CV	8.3	9.1	8.5	8.1	7.8	13.0	5.4	7.5	7.2	12.1	7.6	11.4	10.9	8.6	12.0	6.5	14.9	21.3
	Between Days	StD	35.01	30.73	11.76	10.47	3.55	4.66	57.99	52.53	12.94	6.00	2.62	2.88	62.19	34.38	795	11.40	8.46	9.94
		%CV	5.8	6.4	14.7	10.9	8.2	10.5	6.2	5.0	11.8	14.0	8.1	12.4	7.9	7.9	7.9	8.1	10.8	16.3
Site 2		Mean	559	470	77	88	32	34	905	1000	99	42	18	10	687	425	119	119	35	21
	Within Day 1	StD	22.23	13.77	5.78	4.89	2.56	3.96	30.25	32.93	6.58	5.13	1.39	1.46	20.68	15.72	4.72	4.24	4.75	3.78
		%CV	4.1	3.1	8.4	6.3	8.1	12.5	3.4	3.4	7.6	15.3	7.2	17.9	3.0	4.0	4.3	3.9	14.1	18.5
	Within Day 2	StD	28.30	12.24	3.36	7.02	2.60	2.07	12.96	24.48	2.20	4.32	1.85	2.07	35.62	24.87	4.72	5.70	3.29	3.78
		%CV	4.7	5.9	5.9	8.3	6.8	9.3	1.4	2.5	2.0	10.0	12.0	21.8	5.2	6.2	4.3	4.7	11.5	18.0
	Within Day 3	StD	28.79	28.19	3.62	4.84	2.39	2.00	26.95	28.53	4.57	2.98	1.49	2.07	42.69	20.44	7.45	11.44	6.25	4.12
		%CV	5.0	5.6	4.3	5.0	6.9	5.1	2.9	2.7	4.4	6.0	7.5	16.4	6.2	4.3	5.5	9.1	14.6	18.0
	Between Days	StD	29.97	33.14	7.55	9.62	3.13	4.49	31.76	59.45	10.35	7.84	2.51	2.64	32.78	40.73	13.37	10.22	7.60	3.88
		%CV	5.4	7.1	9.8	10.9	9.8	13.2	3.5	5.9	10.4	18.7	13.8	26.1	4.8	9.6	11.2	8.6	21.7	18.1
Site 3		Mean	584	499	94	143	33	36	931	1046	111	32	17	10	677	457	107	130	31	22
	Within Day 1	StD	27.63	43.55	5.59	7.70	2.67	3.23	20.77	45.47	9.05	5.55	5.37	3.14	26.82	20.59	6.32	5.70	8.67	6.97
		%CV	4.3	7.7	5.3	6.2	7.1	7.8	2.2	4.2	6.5	18.5	27.9	24.4	3.6	3.9	6.6	3.8	20.5	24.5
	Within Day 2	StD	14.48	20.07	6.30	9.57	2.83	4.16	32.04	27.07	8.10	2.60	3.16	2.98	18.56	19.33	8.5	4.49	6.73	9.13
		%CV	2.6	4.3	7.3	6.4	9.3	12.3	3.4	2.6	8.4	7.7	20.5	31.3	2.8	4.6	7.8	3.8	25.2	40.8
	Within Day 3	StD	22.82	17.20	7.69	10.34	2.73	2.97	27.81	43.14	5.42	4.17	2.66	2.49	39.96	16.10	11.08	7.48	5.96	5.76
		%CV	4.1	3.7	8.5	6.6	8.8	9.4	3.1	4.2	5.5	13.3	15.9	32.2	6.4	3.8	9.6	6.2	23.7	34.9
	Between Days	StD	48.55	54.35	10.32	15.98	4.18	5.32	34.56	45.33	21.08	4.39	4.08	3.51	55.59	54.87	12.01	16.52	10.52	8.68
		%CV	8.3	10.9	11.0	11.2	12.7	15.0	3.7	4.3	19.0	13.9	23.8	34.9	8.2	12.0	11.2	12.7	33.5	38.6
Between Sites		StD	42.18	41.99	12.37	27.38	6.21	6.55	45.14	55.95	16.11	8.00	7.60	6.88	70.85	45.63	13.70	15.73	23.07	20.10
		%CV	7.2	8.7	14.8	25.1	17.2	17.3	4.9	5.4	15.1	20.6	33.8	47.7	9.9	10.4	12.6	12.1	47.9	57.5

3. **Cross Reactivity**

To test potential cross reactivity with other IgG antibodies, six specimens were selected that were negative for all EBV markers and were negative for heterophilic and autoimmune antibodies. They were positive for various infectious disease markers, cytomegalovirus (1), herpes simplex virus 1 (3), herpes simplex virus 2 (3), measles (5), mumps (6), rubella (6), toxoplasmosis (1), and varicella zoster virus (5). When these specimens were tested with the ZEUS **AtheNA Multi-Lyte** EBV IgG Plus Test System, they were negative for the EBV markers tested. Specific testing for interference or cross-reactivity with heterophilic and autoimmune antibodies has not been performed.

4. **Potential Interfering Substances**

A study was conducted to determine the potential effects of interfering substances that may be found in serum specimens. The potential interfering substances were spiked into serum specimens at the levels indicated in Table 17.

Table 17: Interfering Substances

Substance	Low Spike	High Spike
Bilirubin	1.9 mg/dL	3.8 mg/dL
Human Albumin	5.5 g/dL	11 g/dL
Human IgG	1.8 g/dL	3.6 g/dL
Cholesterol	200 mg/dL	400 mg/dL
Triglycerides	150 mg/dL	300 mg/dL
Hemoglobin	18 g/dL	360 g/dL
Intralipids	3.5 mg/mL	7 mg/mL

It should be noted that the low and high spiked levels were in addition to the base line level of these materials that may have been present in the original sera. The levels in the original sera were not detected. For this study, for each of the three assays, three EBV IgG positive sera were evaluated in the presence of each of the substances listed above. Two of the sera selected were clearly positive and one of the samples selected was weakly positive. The results of the control specimens and the low and high spiked sera are presented in Table 18.

Table 18: Interfering Substances Specimen Results

Assay	Sample Number		Substance – Spike Level														
			Control – N/A	Bilirubin - Low	Bilirubin - High	Albumin - Low	Albumin - High	IgG - Low	IgG - High	Cholesterol - Low	Cholesterol - High	Triglycerides - Low	Triglycerides - High	Hemoglobin - Low	Hemoglobin - High	Intralipid - Low	Intralipid - High
ZEUS AtheNA EBV IgG Plus	1	Result	711	653	727	631	681	626	705	617	627	581	590	650	641	634	601
		% Positive Signal Recovery		91.8	102.3	88.7	95.8	88.0	99.2	86.8	88.2	81.7	83.0	91.4	90.2	89.2	84.5
	2	Result	581	622	563	578	644	570	597	521	484	534	484	468	544	480	461
		% Positive Signal Recovery		107.1	96.9	99.5	110.8	98.1	102.8	89.7	83.3	91.9	83.3	806	93.6	82.6	79.3
	3	Result	282	287	242	179	174	546	532	220	173	265	194	199	182	199	213
		% Positive Signal Recovery		101.8	85.8	63.5	61.7	193.6	188.7	78.0	61.3	94.0	68.8	70.6	64.5	70.6	75.5
ZEUS AtheNA EBNA IgG Plus	1	Result	994	925	962	931	907	939	945	883	926	861	923	863	909	922	861
		% Positive Signal Recovery		93.1	96.8	93.7	91.2	94.5	95.1	88.8	93.2	86.6	92.9	86.8	91.4	92.8	86.6
	2	Result	859	890	780	799	891	955	1003	734	689	809	682	728	756	715	686
		% Positive Signal Recovery		103.6	90.8	93.0	103.7	111.2	116.8	85.4	80.2	94.2	79.4	84.7	88.0	83.2	79.9
	3	Result	353	380	310	241	226	1034	1007	287	253	342	301	258	242	250	272
		% Positive Signal Recovery		107.6	87.8	68.3	64.0	292.9	285.3	81.3	71.7	96.9	85.3	73.1	68.6	70.8	77.1
ZEUS AtheNA EBV-EA IgG Plus	1	Result	377	337	339	347	432	300	411	297	278	261	266	257	329	290	286
		% Positive Signal Recovery		89.4	89.9	92.0	90.7	79.6	109.0	78.8	73.7	69.2	70.6	68.2	87.3	76.9	75.9
	2	Result	268	258	225	245	281	278	310	229	185	241	175	170	233	175	186
		% Positive Signal Recovery		96.3	84.0	91.4	104.9	103.7	115.7	85.4	69.0	89.9	65.3	63.4	86.9	65.3	69.4
	3	Result	115	102	86	78	57	228	257	75	56	78	74	61	66	83	70
		% Positive Signal Recovery		88.7	74.8	67.8	49.6	198.3	223.5	65.2	48.7	67.8	64.3	53.0	57.4	72.2	60.9

All substances tested showed some level of interference with the specimens using the ZEUS **AtheNA Multi-Lyte** EBV IgG Plus Test System. Recovery of positive signal for low positive Sample 3 ranged from 48.7% to 292.9%, depending on the assay, the interferant identity and level tested (see above).

The unusually high results for the human IgG spiked specimens is likely due to the fact that the human IgG that was spiked was positive for EBV. Specimens that are hemolytic, icteric, lipemic or that contain elevated levels of IgG or cholesterol should not be tested by the ZEUS AtheNA Multi-Lyte EBV IgG Plus Test System. While the limited amount of data presented in the study above may or may not demonstrate it, specimens with elevated levels of these interfering substances may generate false positive or false negative results.

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