EBV-VCA IgM Plus Test System

REF A92101M

IVD

CE

Rx Only

INTENDED USE

The ZEUS **AtheNA Multi-Lyte®** Epstein-Barr Virus - Viral Capsid Antigen (EBV-VCA) IgM Plus Test System is a microparticle-based immunoassay intended for the qualitative detection of IgM class antibody to the Epstein-Barr virus, viral capsid antigen in human serum using the **AtheNA Multi-Lyte** System. The test system is intended to be used for the laboratory diagnosis of EBV-associated infectious mononucleosis and provides epidemiological information on the diseases 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 (EBV) is a ubiquitous human virus which causes infectious mononucleosis (IM), a self limiting lymphoproliferative disease (1). By late adulthood virtually everyone has been infected with 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 manifests 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). In these situations 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).

The presence of antibody to specific EBV antigens correlates with different stages of IM (4, 10 - 12). Both IgM and IgG antibodies to the viral capsid antigen (VCA) peak 3 to 4 weeks after primary EBV infection. IgM anti-VCA decline rapidly and is usually undetectable after 12 weeks. The presence of anti-VCA IgM together with anti-EBNA and anti-EA-R are associated with reactivation of the latent viral carrier state (13, 14).

PRINCIPLE OF THE ASSAY

The ZEUS **AtheNA Multi-Lyte** EBV-VCA IgM Plus Test System is designed to detect IgM class antibodies in human sera to a variety of EBV-VCA antigen. 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 is the EBV viral capsid antigen. 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 IgM is added to the vessel and the plate is incubated. The Conjugate will react with IgM antibody immobilized on the solid phase in step 1. The Bead Suspension is then analyzed by the **AtheNA Multi-Lyte** instrument. The bead set(s) are sorted (identified) and the amount of reporter molecule (PE conjugate) is determined for each bead set. Using the *Intra-Well Calibration Technology®*, internal calibration bead sets are used to convert raw fluorescence into outcome (units).

TEST SYSTEM COMPONENTS

Materials Provided:

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

SOLN	BEAD

1. Bead Suspension: Contains separate distinguishable 5.6 micron polystyrene beads that are conjugated with EBV-VCA gp125 antigen (affinity purified from EBV infected cell lines). The Bead Suspension also contains one bead set designed to detect non-specific and/or RF IgM 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 IgM (μ 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 40-50035).
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 (20).

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-VCA IgM Plus template. Refer to the operators manual for details regarding the operation of the **AtheNA Multi-Lyte** instrument. Results may be read from the filter plate or a reaction plate. **NOTE: For proper specimen analysis, it is important that the instrument is set-up, calibrated and maintained according to the manufacturer's instructions.** Please review the instrument manual for instrument preparation prior to reading the assay results.
12. The plate should be read within 60 minutes after the completion of the Conjugate incubation. One may decide to shake the plate for approximately 15 seconds prior to reading. This optional step may reduce the amount of time required to read the plate.

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

QUALITY CONTROL

Caution: The Negative and Positive Controls are intended to monitor for substantial reagent failure. The Positive Control will not ensure precision at the assay cutoff.

1. Each time the assay is run it is necessary to include the Negative Control (in well A1) and the Positive Control (in well B1).
2. Run validity is determined through the performance of the Positive and Negative Controls. These criteria are analyzed automatically through *Intra-Well Calibration Technology*.
   a. The Negative and Positive Controls must all be negative on the non-specific or control antigen bead.
   b. The Negative Control must be negative for each and every analyte included in the Bead Suspension.
   c. ThePositive Control must be positive for a predetermined group of analytes included in the multiplexed bead suspension. These ranges are lot specific and encoded within the Calibration CD. Positive Control ranges may be viewed by clicking on the "Control Graphs" button of the **AtheNA Multi-Lyte** software and then clicking "Control Upper/Lower Limits".
   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 there is too much activity detected on the non-specific control bead, the specimen results will be invalidated. One probable cause of such a result is the presence of a significant amount of rheumatoid factor IgM antibody in the original sample.
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 (18).

INTERPRETATION OF RESULTS

1. **Calculations**
   a. Assay Calibration: The ZEUS **AtheNA Multi-Lyte** EBV-VCA IgM 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-VCA IgM 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 cutoff for this assay was originally set against 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 included in the kit box.
   b. **EBV Analyte Interpretation**
      i. An **AtheNA Multi-Lyte** result of <100 AU/mL indicates no detectable IgM antibody to EBV-VCA and should be reported as non-reactive.

ii. An **AtheNA Multi-Lyte** result of >120 AU/mL is positive for IgM antibody to EBV-VCA. A positive test result presumes a current or reactivated infection with EBV, and should be reported as reactive for EBV-VCA IgM antibody.

iii. Specimens with AtheNA results in the equivocal range (100 - 120 AU/mL) should be retested in duplicate. Specimens that remain equivocal after repeat testing should be tested by an alternate serologic procedure, such as the ZEUS IFA or ELISA test procedures. Additionally, specimens which remain equivocal after repeat testing should be re-evaluated by drawing another sample one to three weeks later.

iv. If there is too much activity on the NSC (non-specific control) bead*, Intra-Well Calibration Technology* will invalidate that particular specimen. The most likely cause of an invalid specimen in the ZEUS **AtheNA Multi-Lyte** EBV-VCA IgM Test System is due to the presence of RF IgM antibody in the serum sample. For the clinical study, 32 invalid specimens were evaluated for RF IgM antibody. Thirty of the 32 samples were positive for RF IgM. Samples that are INV NSC should be repeated. If they are repeatedly INV NSC, one may test the specimens using another method or may retest the specimens using the modified protocol as outlined below:

1. Obtain ZEUS product number 005M.
2. Begin the assay as outlined above in "Procedure"; however, dilute the repeatedly INV NSC samples in the 005M diluent at a 1:21 dilution.
3. Results may be reported for those specimens that no longer generate INV NSC results.

Step	Optional Assay Procedure for INV NSC Samples
1	Dilute INV NSC specimens 1:21 in 005M Diluent.
2	Incubate dilutions for 30 ± 5 minutes at room temperature.
3	Centrifuge dilutions to pellet the precipitate (5000 – 7000 RPM for 5 – 10 minutes).
4	Combine 50µL of Bead Suspension and 10µL of each dilution in an empty well of a filter plate.
5	Incubate at room temperature for 30 ± 10 minutes.
6	Rinse the microspheres 3 times with 200µL of 1X Wash Buffer.
7	Gently blot the bottom of the plate and air dry for 3 - 5 minutes.
8	Add 150µL of Conjugate to each well. Mix well.
9	Incubate at room temperature for 30 ± 10 minutes.
10	Read results within 60 minutes.

v. The numeric value of the final result above the cutoff is not indicative of the amount of anti-EBV-VCA IgM antibody present.

vi. Most (80%) of IM individuals have peak anti-VCA IgM titers before they consult a physician(4). Therefore, testing paired acute and convalescent sera for significant changes in antibody levels is not useful in most patients with IM (4).

vii. The lack of detectable IgM antibodies does not exclude current EBV infection. The sample may have been collected before development of demonstrable antibody or after the antibody level is no longer detectable.

viii. Specific IgM antibodies are usually detected in patients with recent primary infection, but may be found in patients with reactivated or secondary infections, and they are sometimes found in patients with no other detectable evidence of recent infection.

LIMITATIONS OF THE ASSAY

1. The ZEUS **AtheNA Multi-Lyte** EBV-VCA IgM 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. Specimens with elevated 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. Do not perform testing as a screening procedure for the general population. The predictive value of a positive or negative result depends on the prevalence of analyte in a given patient population. Testing should only be done when clinical evidence suggests the diagnosis of EBV-associated infectious mononucleosis.
5. Performance characteristics of this device have not been established for matrices other than serum.

EXPECTED RESULTS

The clinical study for the product included a total of 693 prospectively collected 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 U.S. Of the 693 specimens tested, 412 included the age and sex of the patient. These included specimens tested at ZEUS and the university medical center. The two hospitals did not include age/sex data with their test results. The ZEUS **AtheNA Multi-Lyte** EBV-VCA IgM Plus results for these 412 specimens by age group and gender are summarized in Table 1.

Table 1: ZEUS AtheNA Multi-Lyte EBV-VCA IgM Plus Results Summary

Age (years)	Gender	Reactive (n)	Non-Reactive (n)	Equivocal (n)	Invalid (n)	Total (n)
1 - 9	Female	0	6	0	1	7
	Male	2	3	0	0	5
	Overall	2	9	0	1	12
10 - 19	Female	17	32	0	0	49
	Male	8	21	1	0	30
	Overall	25	53	1	0	79
20 - 29	Female	23	31	0	3	57
	Male	18	18	0	0	36
	Overall	41	49	0	3	93
30 - 39	Female	3	30	0	1	34
	Male	3	29	0	1	33
	Overall	6	59	0	2	67
40 - 49	Female	0	37	0	3	40
	Male	1	17	0	4	22
	Overall	1	54	0	7	62
50 - 59	Female	1	27	0	1	29
	Male	3	13	0	4	20
	Overall	4	40	0	5	49
60 - 69	Female	0	14	0	5	19
	Male	0	17	0	0	17
	Overall	0	31	0	5	36
70+	Female	0	6	0	2	8
	Male	0	4	0	2	6
	Overall	0	10	0	4	14
Total	Female	44	183	0	16	243
	Male	35	122	1	11	169
	Overall	79	305	1	27	412

Table 2 below shows a breakdown of the patient specimen demographics. The frequency distribution (Figure 1) shows the age distribution of all 412 specimens that included the age of the patient.

Table 2: 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

As with all *in vitro* diagnostic assays, each laboratory should determine its own reference range(s) for the diagnostic evaluation of patient results (19).

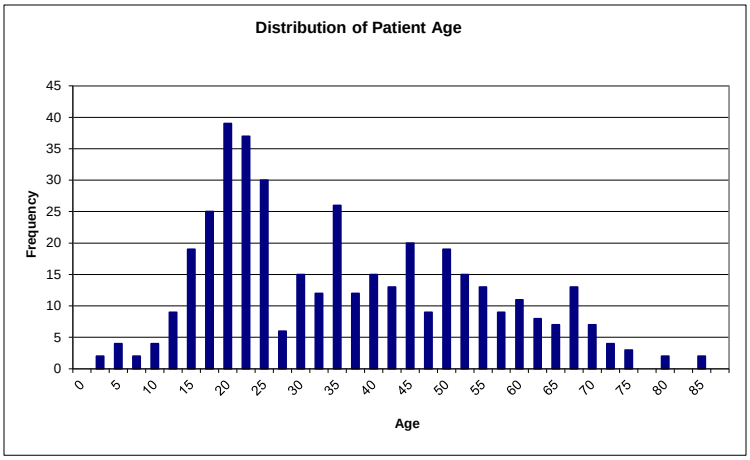

Figure 1: Age Distribution

PERFORMANCE CHARACTERISTICS

1. **Comparative Studies – Results by Specimen Classification**

There were a total of 763 specimens tested. Of the 763, 693 were prospective and 70 were retrospective specimens. The retrospective specimens were purchased as "expected acute" specimens based on clinical signs and symptoms of acute infectious mononucleosis. The actual EBV infection classification of both the prospective and retrospective specimens was determined as described below. The EBV infection classification for each patient in the acute infection, no infection, past infection and indeterminate populations (763 patients total) was determined by a heterophile antibody latex agglutination assay, plus serological assessment using EBV marker profiles obtained from results of commercially available, FDA approved ELISA reference assays. The serological assessment included the following 3 EBV markers: IgG antibodies to Epstein–Barr virus viral capsid antigen (EBV-VCA IgG), IgG antibodies to Epstein–Barr virus nuclear antigen 1 (EBNA-1 IgG), and IgM antibody to Epstein–Barr virus viral capsid antigen (EBV-VCA IgM). The individual ZEUS **AtheNA Multi-Lyte** EBV-VCA IgM Plus assay result was compared to the reference EBV-VCA IgM assay result and to the patient classification. Each patient's EBV infection was classified based on the reactive or nonreactive patterns of the 3 EBV reference serological markers and the heterophile antibody assay. These patterns are presented 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 (-).

Table 4: ZEUS AtheNA Multi-Lyte EBV-VCA IgM Plus Assay versus Comparative EBV-VCA IgM ELISA Assay (Prospective Specimens)

ELISA	Negative				Equivocal				Positive				Total
AtheNA	Reactive	Nonreactive	Equivocal[1]	Invalid	Reactive	Nonreactive	Equivocal[1]	Invalid	Reactive	Nonreactive	Equivocal[1]	Invalid	N
Acute	0	0	0	0	0	0	0	0	24	1	0	3	28
No Infection	0	94	0	1	0	0	0	0	0	0	0	0	95
Past Infection	3	440	1	32	0	4	0	0	0	0	0	0	480
Indeterminate	2	41	0	2	0	0	0	0	18	22	2	3	90
Overall	5	575	1	35	0	4	0	0	42	23	2	6	693

[1] Equivocal results following repeat testing.

Table 5: ZEUS AtheNA Multi-Lyte EBV-VCA IgM Plus Assay versus Comparative EBV-VCA IgM ELISA Assay (Retrospective Specimens)

ELISA	Negative				Equivocal				Positive				Total
AtheNA	Reactive	Nonreactive	Equivocal[1]	Invalid	Reactive	Nonreactive	Equivocal[1]	Invalid	Reactive	Nonreactive	Equivocal[1]	Invalid	N
Acute	0	0	0	0	0	0	0	0	48	0	0	2	50
No Infection	0	1	0	0	0	0	0	0	0	0	0	0	1
Past Infection	0	2	0	0	1	0	0	0	0	0	0	0	3
Indeterminate	0	0	0	0	0	0	0	0	14	2	0	0	16
Overall	0	3	0	0	1	0	0	0	62	2	0	2	70

[1] Equivocal results following repeat testing.

For purposes of percent agreement calculations, the ZEUS **AtheNA Multi-Lyte** EBV-VCA IgM Plus equivocal results (n=3) 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-VCA IgM Plus result. The percent agreement between the ZEUS **AtheNA Multi-Lyte** EBV-VCA IgM Plus assay and the comparative EBV-VCA IgM ELISA assays are summarized in Tables 6 and 7.

Table 6: ZEUS AtheNA Multi-Lyte EBV-VCA IgM Plus Assay versus EBV-VCA IgM Reference ELISA Assay - Prospective Specimens

EBV Classification	Positive % Agreement (x/n)[a]	95% Exact Confidence Interval	Negative % Agreement (x/n)[b]	95% Exact Confidence Interval
Acute	96.0 (24/25/11)	76.9 – 99.9	N/A[c]	N/A
No infection	N/A	N/A	100 (94/94)	96.2 – 100
Past infection	N/A	N/A	99.1 (440/444)	97.7 – 99.7
Indeterminate	42.9 (18/42)	27.7 – 59.0	95.3 (41/43)	84.2 – 99.4
Overall	62.7 (42/67)	50.0 – 74.2	99.0 (575/581)	97.8 – 99.6

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

b x = the number of ZEUS **AtheNA Multi-Lyte** EBV-VCA IgM Plus results that are nonreactive in agreement with the reference EBV-VCA IgM; n = the total number of reference EBV-VCA IgM 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 7: AtheNA Multi-Lyte EBV-VCA IgM Plus Assay versus EBV-VCA IgM Reference ELISA Assay - Retrospective Specimens (Expected Acute)

EBV Classification	Positive % Agreement (x/n)[a]	95% Exact Confidence Interval	Negative % Agreement (x/n)[b]	95% Exact Confidence Interval
Acute	100 (48/48)	92.6 - 100	N/A[c]	N/A
No infection	N/A	N/A	100 (1/1)	N/A
Past infection	N/A	N/A	100 (2/2)	15.8 – 100
Indeterminate	87.5 (14/16)	61.7 – 98.4	N/A	N/A
Overall	96.9 (62/64)	89.2 – 99.6	100 (3/3)	29.2 – 100

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

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

Assay precision was evaluated at multiple sites as follows: six samples were identified for use in the study based upon their activity on the ZEUS **AtheNA Multi-Lyte** EBV-VCA IgM Plus Test System. Two samples were selected that were clearly negative, two that were clearly positive and two samples that were near the assay cut off. This panel of six serum samples were split into three aliquots each and tested at three clinical sites. One 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 performed on three days at each facility. A summary of this testing appears in Table 8.

Table 8: ZEUS AtheNA Multi-Lyte EBV-VCA IgM Plus Precision Testing

			Panel 1	Panel 2	Panel 3	Panel 4	Panel 5	Panel 6
Site 1		Mean	19	15	902	435	151	192
	Within Day 1	StD	1.66	1.16	35.65	26.39	11.25	13.81
		%CV	8.7	8.2	3.9	5.9	7.2	7.2
	Within Day 2	StD	2.51	1.73	52.15	19.63	10.91	13.97
		%CV	13.2	10.2	5.7	4.6	7.6	7.1
	Within Day 3	StD	1.55	1.16	44.51	19.78	7.69	10.01
		%CV	7.8	7.9	5.0	4.6	5.0	5.4
	Between Days	StD	1.91	1.76	45.48	22.58	10.69	13.00
		%CV	9.8	11.5	5.0	5.2	7.1	6.8
Site 2		Mean	13	16	836	391	130	167
	Within Day 1	StD	1.67	1.04	45.24	35.43	8.75	10.56
		%CV	12.6	6.0	5.2	8.7	6.1	5.8
	Within Day 2	StD	1.85	1.69	46.84	28.72	5.45	15.33
		%CV	16.1	11.7	5.5	7.4	4.5	9.4
	Within Day 3	StD	2.79	2.75	40.10	40.09	14.41	15.34
		%CV	20.8	17.3	5.0	10.6	11.7	9.7
	Between Days	StD	2.13	2.22	51.80	35.35	14.18	16.86
		%CV	16.9	14.0	6.2	9.0	10.9	10.0
Site 3		Mean	14	17	897	427	139	181
	Within Day 1	StD	2.00	1.77	20.49	33.20	7.84	9.02
		%CV	14.8	10.6	2.4	8.5	6.1	5.3
	Within Day 2	StD	1.07	1.49	47.06	29.23	14.82	12.75
		%CV	7.9	8.4	5.1	6.5	10.0	7.0
	Within Day 3	StD	2.17	2.00	63.53	28.63	8.38	6.79
		%CV	15.6	11.8	6.9	6.5	6.0	3.5
	Between Days	StD	1.74	1.75	56.76	38.51	12.82	13.40
		%CV	12.8	10.2	6.3	9.0	9.2	7.4
Between Sites		StD	3.55	2.04	59.13	37.69	15.21	17.49
		%CV	23.2	12.7	6.7	9.0	10.9	9.7

3. **Cross Reactivity**

The ZEUS **AtheNA Multi-Lyte** EBV-VCA IgM Plus Test System was evaluated for potential cross reactivity to other antibodies. For this study, a total of 30 specimens were evaluated. Thirteen of the specimens were positive for IgM antibody to other infectious disease agents (Cytomegalovirus, Herpes Simplex Virus, Rubella and Toxoplasma). Of the 13 specimens evaluated, none were reactive on the ZEUS **AtheNA Multi-Lyte** EBV-VCA IgM Plus assay. Fourteen specimens were tested that possessed various autoantibodies to nuclear antigens. Of the 14 specimens tested, none of them were reactive on the ZEUS **AtheNA Multi-Lyte** EBV-VCA IgM assay. Finally, four specimens were tested that were found to be RF IgM positive. All four specimens yielded invalid results on the ZEUS **AtheNA**

Multi-Lyte EBV-VCA IgM Plus assay. These specimens were invalidated by Intra-Well Calibration Technology®, since the non-specific bead contained in the Bead Suspension is designed to detect such activity and invalidate the specimens.

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 9.

Table 9: 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	7.0 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-VCA IgM 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 reactive. The results of the control specimens and the low and high spiked sera are presented in Table 10.

Table 10: Interfering Substances Specimen Results

		Sample 1		Sample 2		Sample 3	
Substance	Spike Level	VCA IgM Strong Positive	% Positive SignalRecovered	VCA IgM Strong Positive	% Positive SignalRecovered	VCA IgM Weak Positive	% Positive SignalRecovered
Control	N/A	**777**		**719**		**246**	
Bilirubin	Low	881	113.4	716	99.6	208	84.6
	High	874	112.5	540	75.1	176	71.5
Albumin	Low	706	90.9	688	95.7	2204	82.9
	High	742	95.5	678	94.3	177	72.0
IgG	Low	749	96.4	579	80.5	135	54.9
	High	665	85.6	511	71.1	89	36.2
Cholesterol	Low	838	107.9	780	108.5	185	75.2
	High	817	105.1	658	91.5	205	83.3
Triglycerides	Low	847	109.0	603	83.9	181	73.6
	High	808	104.0	555	77.2	136	55.3
Hemoglobin	Low	752	96.8	575	80.0	173	70.3
	High	803	103.3	658	91.5	128	52.0
Intralipid	Low	780	100.4	781	108.6	211	85.8
	High	782	100.6	674	93.7	216	87.8

All substances tested showed some level of interference with detection of low positive EBV-VCA IgM specimens using the ZEUS **AtheNA Multi-Lyte** EBV-VCA IgM Plus Test System. Recovery of positive signal for low positive Sample 3 ranged from 36.2% to 87.8%, depending on the interferant identity and level tested (see above). Specimens that are hemolytic, icteric, lipemic or that contain elevated levels of IgG should not be tested by the ZEUS **AtheNA Multi-Lyte** EBV-VCA IgM Plus Test System.

REFERENCES

1. Rapp CE, and Heweston JF: Infectious mononucleosis and the Epstein-Barr virus. Am. J. Dis. Child. 132:78, 1978.
2. Biggar RJ, Henle W, Fleisher G, Bocker J, Lennette ET, and Henle G: Primary Epstein-Barr virus infections in African infants. I: Decline of maternal antibodies and time of infection. Int. J. Cancer. 22:239, 1978.
3. Fry J: Infectious mononucleosis: Some new observations from a 15 year study. J. Fam. Prac. 10:1087, 1980.
4. Lennette ET: Epstein-Barr virus. In: Manual of Clinical Microbiology, 4th edition. Lennette ET, Balows A, Hausler WJ, Shadomy HJ, eds. Washington DC, American Society for Microbiology, p. 326, 1987.
5. Fleisher G, Henle W, Henle G, Lennette ET, and Biggar RJ: Primary infection with Epstein-Barr virus in infants in the United States: Clinical and Serological Observations. J. Infect. Dis. 139:553, 1979.
6. Merlin TL: Chronic mononucleosis: Pitfalls in the laboratory diagnosis. Hum. Path. 17:2, 1986.
7. Sixbey JW, Nedrud JG, Raab-Traub N, Hanes RA, Pagano JS: Epstein-Barr virus replication in oropharyngeal epithelial cells. New Eng. J. Med. 310:1225, 1984.
8. Chang RS, Lewis JP, Reynolds RD, Sullivan MJ, Neuman J: Oropharyngeal excretion of Epstein-Barr virus by patients with lymphoproliferative disorders and by recipients of renal homografts. Ann. Intern. Med. 88:34, 1978.
9. Jones JF, Ray G, Minnich LL, Hicks MJ, Kibler R, Lucus DO: Evidence of active Epstein-Barr virus infection in patients with persistent, unexplained illness. Elevated anti-early antigen antibodies. Ann. Intern. Med. 102:1, 1985.
10. Evans AS, Neiderman JC, Cenabre LC, West B, and Richards VA: A prospective evaluation of heterophile and Epstein-Barr virus-specific IgM antibody tests in clinical and subclinical infectious mononucleosis: Specificity and sensitivity of the tests and persistence of antibody. J. Infect. Dis. 132:546, 1975.
11. Henle W, Henle GE, and Horowitz CA: Epstein-Barr virus specific diagnostic tests in infectious mononucleosis. Hum. Path. 5:551, 1974.
12. Lennette ET, and Henle W: Epstein-Barr virus infections: Clinical and serological features. Lab Management. p. 23, June, 1987.
13. Horowitz CA, Henle W, Henle G, and Schmitz H: Clinical evaluation of patients with infectious mononucleosis and development of antibodies to the R component of the Epstein-Barr virus-induced early antigen complex. Am. J. Med. 58:330, 1975.
14. Sumaya CV: Endogenous reactivation of Epstein-Barr virus infections. J. Infect. Dis. 135:374, 1977.
15. U.S. Department of Health and Human Services. Public Health Service. Centers for Disease Control and Prevention and National Institutes of Health. U.S. Government Printing Office, Washington D.C., 4th Ed., 1999.

16. U.S. Department of Labor, Occupational Safety and Health Administration; Occupational Exposure to Bloodborne Pathogens, Final Rule. Fed.Register 56:64175-64182, 1991.
17. Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids and Tissues; Approved Guideline. NCCLS Document M29, Vol.17(12), 1997.
18. CLSI. *User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline*. CLSI document EP12-A. CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2002.
19. CLSI. *How to define, determine, and utilize reference intervals in the clinical laboratory; approved guideline*. CLSI Document C28-A. CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA;1995.
20. Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guidelines – 4th Edition (2010). CLSI Document GP44-A4 (ISBN 1-56238-724-3). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, PA 19087.

ZEUS Scientific

ZEUS Scientific
200 Evans Way, Branchburg, New Jersey, 08876, USA
Toll Free (U.S.): 1-800-286-2111, Option 2
International: +1 908-526-3744
Fax: +1 908-526-2058
Website: www.zeusscientific.com

In the US & Canada, call toll free, or e-mail Customer Service (orders@zeusscientific.com) or Technical Service (support@zeusscientific.com).
For all other countries, please contact your local distributor.

EC REP
EMERGO EUROPE
Westervoortsedijk 60
6827 AT Arnhem
The Netherlands