MMV IgG Plus Test System

REF A93111G

IVD

CE

Rx Only

INTENDED USE

The ZEUS **AtheNA Multi-Lyte®** MMV IgG Plus Test System is intended for the qualitative presumptive detection of IgG class antibody to Measles (Rubeola), Mumps, and Varicella-Zoster (VZ) viruses in human serum using the AtheNA Multi-Lyte® System. The test is intended to be used for determination of a previous infection with the Measles, Mumps, VZ viruses and to determine the serological status of individuals including women of childbearing age. Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients, cord blood, neonatal specimens, infants or children. This test is for *In Vitro* diagnostic use only.

SIGNIFICANCE AND BACKGROUND

Measles is a highly contagious viral disease resulting from infection with a paramyxovirus (genus *Morbillivirus*). Eight to 12 days following infection, a prodromal phase of Measles begins which is marked by fever, cough, coryza, and possibly conjunctivitis. In many cases, the onset of the prodromal symptoms is followed within two to three days by the appearance of a specific enenthem (Koplik's spots) and a generalized maculopapular eruption (three to four days after onset) (1). In uncomplicated Measles, the appearance of the rash is followed by a peak in temperature one to two days later, and a rapid defervescence on the third or fourth day of the rash.

Under normal circumstances, the appearance of the prodromal symptoms, especially the highly specific and pathognomonic Koplik's spots, is sufficient for clinical diagnosis. Since the introduction of the Measles vaccine in 1963 however, the incidence of Measles has dramatically decreased (2). As a result, medical professionals have had less experience in the clinical diagnosis of the disease and may require laboratory assistance for confirmation.

Diagnosis of Measles can be further complicated by the appearance of an atypical form in persons who were immunized with an inactivated Measles vaccine between 1963 and 1967, and were subsequently re-infected with the wild-type virus (3). The atypical form of measles may be severe and clinically confused with Rocky Mountain Spotted Fever. In addition, acute Measles may be complicated by secondary bacterial infections of the respiratory tract and middle ear. Additional complications may include a post-infectious encephalitis and a rare, but often times fatal disease, subacute sclerosing panencephalitis (SSPE) (1).

Antibodies to the Measles virus begin to appear with the development of the rash. A transient IgM antibody response (three to six weeks) may appear first, or in conjunction with IgG. IgG antibodies peak in two to six weeks, decline gradually over six months, and remain relatively stable thereafter. Following the administration of live, attenuated Measles vaccine, the antibody can be detected 11 - 14 days after inoculation (1). Subclinical re-infections can occur in persons with either vaccine-induced or natural immunity resulting in a boost in Measles-specific IgG titer (1). In spite of the wide-spread vaccination program, many individuals remain susceptible to Measles as a result of primary vaccine failure, or non-immunization. Serology is a useful tool for ascertaining the immune status of previously vaccinated individuals and detection of seroconversion in recently vaccinated individuals. In addition, Measles serology can be a valuable tool in the diagnosis of SSPE, which may occur years after the original measles infection (3).

Mumps is an acute, generally self-limiting, contagious disease with moderate fever of short duration. Bilateral or unilateral parotitis is the most common clinical feature. Secondary involvement concerns the testes, ovaries, central nervous system and more rarely, the pancreas, peripheral nerves, eye, inner ear and other organs (4).

The incubation period for Mumps Virus ranges between 18 and 21 days. Infections are spread by droplets via the upper respiratory route. Between 25 and 50 percent of all infections are silent. Immunity after infection appears to be lifelong; however, silent re-infections may occur although it is probably an infrequent event. An attenuated live virus vaccine is available which induces lower levels of measurable antibody than natural infection (4, 5). Only one distinct antigenic type of Mumps virus is known. Some antigenic cross reactivity and anamnestic antibody responses exist with other paramyxoviruses, particularly Parainfluenza Type 1, in some serological tests (4, 5, and 6).

Many tests for the determination of antibodies to Mumps virus have been described. The traditional assays of viral neutralization, hemagglutination inhibition (HI) and complement fixation (CF), all have the drawbacks of either being too cumbersome for routine serological work, or have shortcomings with regard to sensitivity and reliability. Both CF and HI suffer from relatively low sensitivity and cross reacting antibodies to other paramyxoviruses may pose a problem (4, 5). Both IFA and ELISA tests have the advantages of being sensitive and capable of allowing the separate identification of IgG and IgM viral antibodies for bith determination of immune status and diagnosis of acute infection (4, 5).

Varicella-Zoster virus (VZV) is a common pathogen of humans. The clinical course of VZV in humans is generally categorized into Varicella (chickenpox) and *Herpes zoster* (shingles). The major significant advance in understanding the nature of these agents was originally contributed by Weller and co-workers who demonstrated the method for isolation and serial propagation of the virus (7, 8), and more recently, the epidemiology and control of the virus (9). Viral isolates obtained from patients with chickenpox and zoster were demonstrated to be identical on the basis of cytopathic effect (26), antigenicity (8), and morphology (10, 11). More recently, these viruses have been shown to have identical DNA molecular weight (12), and restriction endonuclease patterns (13).

The clinical symptoms of primary Varicella (chickenpox) include a prodromal period of headaches, malaise, and fever preceding the exanthem, or the characteristic eruptions may be the first symptom. The rash is pleomorphic and goes through evolution from macular to papular, and then to vesicular stage. The rash characteristically develops in successive crops of new lesions over a 3 - 5 day period.

Chickenpox normally infects children in primary school . Adults, adolescents, and newborns are also susceptible to infection. The disease usually appears in the winter or spring, and may reach epidemic levels in a susceptible population. Varicella infections during early pregnancy rarely have been found to cause congenital anomalies. Varicella infections occurring in susceptible pregnant women at the time of delivery may have a life-threatening infection in the newborn, as well as patients in a variety of pathologies (14, 15, and 16). The potential spread of a nosocomial disease is not uncommon.

Herpes zoster (shingles) is a disease primarily of adults, with most of the cases occurring in the age group over 50 years. In contrast to the epidemic and seasonal nature of Varicella infection, *Herpes zoster* has a random pattern of occurrence. *Herpes zoster* is believed to be the re-activation of a pre-existing Varicella virus which has been in a latent state since the occurrence of primary Varicella infection. Persons affected with *Herpes zoster* infections do so even in the presence of pre-existing antibody levels to Varicella virus. Symptoms of *Herpes zoster* are erythematous, maculopapular areas which develop over an area of skin served by an afferent nerve. Single, or clumps of, vesicles then appear, usually accompanied by pain which, in some cases, can be extreme (17).

Based on the epidemiologic evidence that VZV is spread by droplet nuclei or air droplets, and possibly by skin squames, the portal of entry of the virus is assumed to be through the respiratory passages (18). After dissemination of VZV from the blood, it rapidly spreads to the skin and is detectable in the endothelium, and then involves the cells of the epidermis with accumulation of fluid between the prickle cell layer and outer epidermis forming a vesicle (19). The vesicle becomes the site of intense immunologic activity with initial infiltration of polymorphonuclear leukocytes that remain the predominant inflammatory cell as observed in *Herpes zoster* (20). Later, mononuclear cells migrate into the vesicle.

PRINCIPLE OF THE ASSAY

The ZEUS **AtheNA Multi-Lyte** MMV IgG Plus Test System is designed to detect IgG class antibodies in human sera to Measles (Rubeola), Mumps, and VZV. 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); in this case, there are three primary bead sets, Measles, Mumps, and VZV. 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®.**

SOLN	BEAD

1. Bead Suspension: Contains separate distinguishable 5.6 micron polystyrene beads that are conjugated with Rubeola antigen (partially purified Edmonston strain from vero cells), Mumps antigen (partially purified Enders strain from LLC-MK2 cells), and VZV antigen (partially purified Ellen strain from human fibroblasts). 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 (21, 22).
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) (23).
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 (26).

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 MMV 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.

INTERPRETATION OF RESULTS

1. **Calculations**
   a. Assay Calibration: The ZEUS **AtheNA Multi-Lyte** MMV 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** MMV 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 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.
   b. **Measles, Mumps and VZV Analytes Interpretation**: Specimen unit values for the analyte is interpreted as follows:
      i. An **AtheNA Multi-Lyte** result of <100 AU/mL indicates no detectable IgG antibody to Measles, Mumps or VZV - report as non-reactive to IgG antibodies.
      ii. An **AtheNA Multi-Lyte** result of >120 AU/mL is presumptive positive for IgG antibody to Measles Mumps or VZV. A positive test result presumes a current or past infection, or prior immunization to Measles Mumps or VZV - report as presumptive positive for IgG antibodies.
      iii. Re-test specimens with **AtheNA Multi-Lyte** results in the equivocal range (100 - 120 AU/mL) in duplicate. Test repeatedly equivocal specimens by an alternate serologic procedure, such as the ZEUS ELISA test procedure. Additionally, re-evaluate repeatedly equivocal specimens 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.
      v. The numeric value of the final result above the cutoff is not indicative of the amount of anti-IgG antibody present. Significant antibody increases between acute and convalescent specimens may not be determined.
      vi. A negative test result does not preclude immunity to Measles, Mumps or VZV infection. In some patients levels of the IgG antibody may fall below the detection limit of this assay.

LIMITATIONS OF THE ASSAY

1. The ZEUS **AtheNA Multi-Lyte** MMV IgG Plus Test System is a diagnostic aid and by itself is not diagnostic. Test results should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
2. Performance characteristics of this device have not been established with syphilis-associated disease.
3. Do not perform testing as a screening procedure for the general population. The predictive value of a positive or negative result depends on the prevalence of analyte in a given patient population.
4. Hemolytic, icteric, or lipemic samples may interfere with the outcome of this assay. Additionally, specimens with abnormal IgG and RF antibody concentrations may interfere with the outcome of this assay. Avoid the use of these types of specimens.
5. Test results of specimens from immunosuppressed patients may be difficult to interpret.
6. Performance characteristics of this device have not been established for matrices other than serum.
7. Performance characteristics of this device have not been established with specimens containing heterophile antibodies which are known to cause false positive results in various immunoassays.
8. The performance characteristics have not been established with vaccine recipients to determine if the assay will detect an immune response to a vaccine.
9. A single positive result only indicates previous immunologic exposure; level of antibody response or class of antibody response may not be used to determine

active infection or disease stage.

10. Samples collected too early in the course of an infection may not have detectable levels of IgG. In such cases, a second sample may be collected after two to seven weeks and tested concurrently with the original sample to look for seroconversion.
11. Positive results from patients who have received blood products within the previous six months may be due to transient antibody levels acquired during transfusion.
12. Usage for cord blood, the neonatal population and pre-transplant patients has not been established.

EXPECTED RESULTS

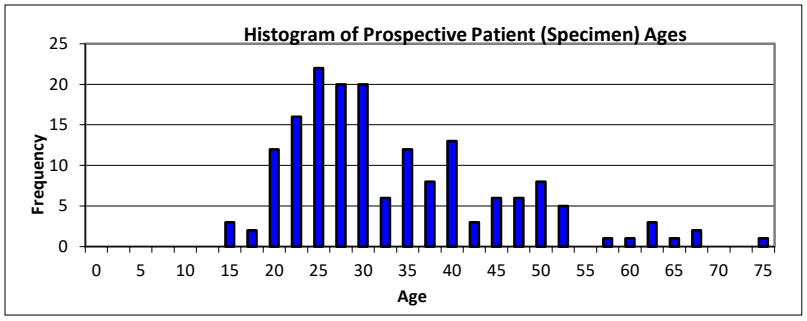
The clinical study for these analytes included a total of 177 prospectively collected specimens. These specimens were tested at a centralized hospital laboratory located in Southeastern US and in a reference laboratory located in the Midwest. Of the 177 prospective specimens, 171 were supplied with both the age and sex of the patient. The **AtheNA Multi-Lyte** results for these 171 specimens by age group and gender are summarized in the following table.

		AtheNA Multi-Lyte Measles Plus Analyte				AtheNA Multi-Lyte Mumps Plus Analyte				AtheNA Multi-Lyte VZV Plus Analyte			
Age	**Sex**	**Positive**	**Negative**	**Equivocal**	**Invalid**	**Positive**	**Negative**	**Equivocal**	**Invalid**	**Positive**	**Negative**	**Equivocal**	**Invalid**
1 - 9	Male	0	0	0	0	0	0	0	0	0	0	0	0
	Female	0	0	0	0	0	0	0	0	0	0	0	0
10 – 19	Male	5	0	0	0	6	1	0	0	2	4	0	0
	Female	5	1	1	0	4	0	1	0	3	3	0	0
20 - 29	Male	17	2	2	0	15	0	3	1	18	0	1	1
	Female	36	13	6	1	45	10	3	0	45	11	2	0
30 – 39	Male	1	1	2	0	3	0	1	0	4	0	0	0
	Female	22	10	3	0	23	12	1	0	31	4	1	0
40 – 49	Male	1	3	0	0	1	0	1	0	4	0	0	0
	Female	18	0	2	0	21	2	0	0	20	0	0	0
50 - 59	Male	4	0	0	0	2	0	0	0	3	1	0	0
	Female	4	2	0	0	7	0	1	0	5	1	0	0
60 - 69	Male	0	0	0	0	0	0	0	0	0	0	0	0
	Female	6	0	0	0	6	0	0	0	4	1	1	0
70+	Male	0	0	0	0	0	0	0	0	0	0	0	0
	Female	1	0	0	0	1	0	0	0	1	0	0	0
Total	Male	28	6	4	0	27	1	5	1	31	5	1	1
	Female	94	26	12	1	107	24	6	0	109	20	4	0
	Total	171	32	16	1	134	25	11	1	140	25	5	1

With the exception of missing ages for four of the 177 samples and missing sex from two of the 177, all prospective specimens (n = 171) were supplied with the age and sex from the individual that the specimen was obtained. A summary of this demographic information appears in the table below:

Statistic	**Females**	**Males**	**Total Population**
Sample Size	133	38	171
Mean Age	33.4	29.3	32.5
Median Age	30	26.5	28
Minimum Age	15	14	14
Maximum Age	73	52	73

Below is a histogram of the frequency of the age of all 171 specimens for which the data was available.



PERFORMANCE CHARACTERISTICS

1. **Measles Analyte**

a. **A comparative study** was conducted where a total of 253 specimens were tested. Of the 253 specimens tested, 177 were prospective specimens and 76 were retrospective specimens. The prospective specimens were tested at a centralized hospital laboratory located in Southeastern US and in a reference laboratory located in the Midwest. The 76 retrospective specimens were comprised of 76 pregnant women ranging in age from 18 to 41. Of the 76 pregnant women, 16/76 were in their first trimester of pregnancy, 30/76 were in their second trimester and 30/76 were in their third trimester of pregnancy. Specimens were tested using the **AtheNA Multi-Lyte** MMV IgG Plus Test System and the ZEUS ELISA Measles IgG Test System. The results of this comparative study are depicted below in Tables 1 - 5:

Table 1: Site One Prospective Samples

		ZEUS ELISA Measles IgG Test System Results			
		Positive	Negative	Equivocal	Total
AtheNA Multi-Lyte MMV IgG Plus Test System	Positive	80	0	0	80
	Negative	2	0	0	2
	Equivocal	5	0	0	5
	Invalid	0	0	0	0
	Total	87	0	0	87
Positive % Agreement = 80/82 = 95.3%			95% Confidence Interval – 90.9 to 99.8%		
Negative % Agreement = 0/0 = N/A			95% Confidence Interval – N/A		
Overall Agreement = 80/87 = 92.0%			95% Confidence Interval – 86.2 to 97.7%		

Table 2: Site Two Prospective Samples

		ZEUS ELISA Measles IgG Test System Results			
		Positive	Negative	Equivocal*	Total
AtheNA Multi-Lyte MMV IgG Plus Test System	Positive	48	0	0	48
	Negative	12	17	4	33
	Equivocal*	4	2	2	8
	Invalid**	1	0	0	1
	Total	65	19	6	90
Positive % Agreement = 48/82 = 75.0%			95% Confidence Interval – 64.4 to 85.6%		
Negative % Agreement = 17/19 = 89.5%			95% Confidence Interval – 75.7 to 103.3%		
Overall Agreement = 65/89 = 73.0%			95% Confidence Interval –63.8 to 82.3%		
*AtheNA and ELISA specimens showing equivocal results were considered to be as "non-agreement" specimens.					
**Specimens showing invalid results were excluded from calculations for agreement.					

Table 3: Combined Sites Prospective Samples

		ZEUS ELISA Measles IgG Test System Results			
		Positive	Negative	Equivocal*	Total
AtheNA Multi-Lyte MMV IgG Plus Test System	Positive	128	0	0	128
	Negative	14	17	4	35
	Equivocal*	9	2	2	13
	Invalid**	1	0	0	1
	Total	152	19	6	177
Positive % Agreement = 128/155 = 82.6%			95% Confidence Interval – 76.6 to 88.6%		
Negative % Agreement = 17/19 = 89.5%			95% Confidence Interval – 75.7 to 103.3%		
Overall Agreement = 145/176 = 82.4%			95% Confidence Interval –76.8 to 88.0%		
*AtheNA and ELISA specimens showing equivocal results were considered to be as "non-agreement" specimens.					
**Specimens showing invalid results were excluded from calculations for agreement.					

Table 4: Retrospective Samples

		ZEUS ELISA Measles IgG Test System Results			
		Positive	Negative	Equivocal	Total
AtheNA Multi-Lyte MMV IgG Plus Test System	Positive	71	0	0	71
	Negative	4	1	0	5
	Equivocal	0	0	0	0
	Invalid	0	0	0	0
	Total	75	1	0	76
Positive % Agreement = 71/75 = 94.7%			95% Confidence Interval – 89.6 to 99.8%		
Negative % Agreement = 1/1 = 100.0%			95% Confidence Interval – 75.7 to 103.3%		
Overall Agreement = 72/76 = 94.7%			95% Confidence Interval – 89.7 to 99.8%		

Table 5: All Samples Combined

		ZEUS ELISA Measles IgG Test System Results			
		Positive	Negative	Equivocal*	Total
AtheNA Multi-Lyte MMV IgG Plus Test System	Positive	199	0	0	199
	Negative	18	18	4	40
	Equivocal*	9	2	2	13
	Invalid**	1	0	0	1
	Total	227	20	6	253
Positive % Agreement = 199/227 = 87.7%			95% Confidence Interval – 83.4 to 91.9%		
Negative % Agreement = 18/20 = 90.0%			95% Confidence Interval – 76.9 to 103.1%		
Overall Agreement = 217/252 = 86.1%			95% Confidence Interval –81.8 to 90.4%		
*AtheNA and ELISA specimens showing equivocal results were considered to be as "non-agreement" specimens.					
**Specimens showing invalid results were excluded from calculations for agreement.					

b. **Assay precision and reproducibility** were evaluated at multiple sites as follows: six samples were identified for use in the study based upon their activity on the **AtheNA Multi-Lyte** MMV IgG 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 the 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. The summary of the precision study appears in Table 6.

Table 6: Measles Precision

		Intra-Assay									Inter-Assay			Between Sites
		Site One			Site Two			Site Three						
Sample		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Site One	Site Two	Site Three	
1	Mean	324.5	319.5	311.5	343.8	356.8	323.3	359.3	351.1	352.0	318.5	341.3	354.1	338
	StD	29.7	12.0	16.4	19.8	20.5	19.1	14.8	23.0	16.4	20.6	23.6	18.0	25.4
	% CV	9.2	3.7	5.3	5.8	5.8	5.9	4.1	6.5	4.7	6.5	6.9	5.1	7.5
2	Mean	153.1	152.5	144.0	167.8	154.4	141.9	186.1	185.4	177.0	149.9	154.7	182.8	162.5
	StD	5.6	14.7	10.7	8.5	4.8	9.9	15.9	15.5	9.8	11.3	13.2	14.1	19.4
	% CV	3.6	9.7	7.5	5.1	3.1	7.0	8.6	8.4	5.6	7.6	8.6	7.7	11.9
3	Mean	479.1	472.5	464.0	481.0	492.3	468.4	538.5	541.4	530.3	471.9	48.5	536.7	496.4
	StD	26.0	23.7	25.2	92.2	29.6	17.2	44.3	54.8	33.2	24.7	55.2	43.2	51.3
	% CV	5.4	5.0	5.4	19.2	6.0	3.7	8.2	10.1	6.3	5.2	11.5	8.1	10.3
4	Mean	59.0	51.3	47.9	53.9	61.1	41.3	77.3	77.8	76.1	52.7	52.1	77.0	60.6
	StD	6.4	4.5	4.7	6.0	3.9	7.0	4.2	6.0	3.8	6.9	10.1	4.6	13.9
	% CV	10.8	8.7	9.8	11.1	6.5	17.1	5.4	7.7	5.0	13.1	19.3	6.0	22.9
5	Mean	29.6	20.4	22.8	29.6	38.0	20.1	57.8	48.3	50.4	24.3	29.3	52.1	35.2
	StD	7.5	4.5	6.4	7.5	6.6	6.4	19.1	5.0	4.3	7.2	9.9	11.9	15.6
	% CV	25.4	22.3	28.1	25.3	17.3	32.0	33.0	10.4	8.5	29.7	33.9	22.8	44.4
6	Mean	141.8	126.9	121.0	138.8	149.4	135.8	172.1	169.8	172.6	129.9	141.3	171.5	147.6
	StD	6.5	46.8	9.5	10.2	10.3	16.2	6.5	11.4	8.8	28.0	13.4	8.8	25.5
	% CV	4.6	36.9	7.8	7.3	6.9	12.0	3.8	6.7	5.1	21.6	9.5	5.1	17.3

c. **Cross reactivity** testing was conducted using seven serum specimens that were negative on the AtheNA Multi-Lyte® MMV IgG Plus Test System and were subsequently tested by commercially available ELISAs for activity to HSV-1, HSV-2, Toxo, CMV, EBV Nuclear Antigen, EBV EA IgG and EBV Viral Capsid Antigen. Five of the seven samples were positive for one or more of the viral markers tested. The results of this study appear in Table 7.

Table 7: Measles Cross Reactivity

	AtheNA Multi-Lyte Results	ELISA Results						
Sample		CMV	HSV-1	HSV-2	Toxoplasma	EBNA	EBV-EA IgG	EBV
CN 18	45	0.10	5.46	1.22	0.01	6.44	0.18	5.89
CN 126	96	1.83	0.38	0.21	0.03	0.08	0.63	2.15
CN 140	50	1.88	1.06	0.16	0.00	3.14	0.20	0.82
CN 141	81	2.41	3.08	0.62	0.05	5.46	0.24	2.97
CN 144	70	1.75	0.87	0.14	0.02	2.00	0.21	0.92
CN 146	42	1.92	1.31	0.36	0.05	3.83	0.84	1.42
CN 149	25	1.52	0.38	0.22	0.04	6.58	0.39	1.33

d. **An interfering substances** study was conducted to determine the potential effects of interfering substances that may be found in serum specimens. The following potentially interfering substances were spiked into serum specimens at the levels indicated in Table 8 below:

Table 8: Measles Interfering Substances Levels

Substance	Low Spike	High Spike
Bilirubin	1.9mg/dL	3.8mg/dL
Human Albumin	5.5g/dL	11g/dL
Human IgG	1.8g/dL	3.6g/dL
Cholesterol	200mg/dL	400mg/dL
Triglycerides	150mg/dL	300mg/dL
Hemoglobin	180g/dL	360g/dL
Intralipids	3.5mg/mL	7.0mg/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, three sera were evaluated in the presence of each of the substances above. One specimen was clearly positive for Measles IgG, one was borderline and one was clearly negative for IgG. The results of the control specimens and the low and high spiked sera are presented in Table 9 below:

Table 9: Measles Interfering Substance Results

		Sample One		Sample Two		Sample 3	
Interfering Substance	**Spike Level**	**Measles Positive**	**% Positive Signal Recovered**	**Measles Equivocal**	**% Positive Signal Recovered**	**Measles Negative**	**% Positive Signal Recovered**
None (Control)	N/A	382	N/A	102	N/A	24	N/A
Bilirubin	Low	426	111.5	130	127.5	25	104.2
Bilirubin	High	415	108.6	109	106.9	22	91.7
Albumin	Low	453	118.6	100	98.0	26	108.3
Albumin	High	440	115.2	103	101.0	24	100.0
IgG	Low	477	124.9	297	291.2	328	1366.7
IgG	High	545	142.7	359	352.0	365	1520.8
Cholesterol	Low	424	111.0	107	104.9	22	91.7
Cholesterol	High	414	108.4	115	112.7	27	112.5
Triglycerides	Low	403	105.5	112	109.8	23	95.8
Triglycerides	High	388	101.6	110	107.8	28	116.7
Hemoglobin	Low	388	101.6	102	100.0	27	112.5
Hemoglobin	High	372	97.4	95	93.1	16	66.7
Intralipid	Low	389	101.8	121	118.6	29	120.8
Intralipid	High	616	161.3	106	103.9	25	104.2

The positive sample showed a range of recovery from 161.3% with the high spike of Intralipid to a low of 97.4% with the high spike of hemoglobin. The addition of purified IgG also caused a significant rise in signal since it is likely the purified human IgG used to spike the specimen was positive for anti-Measles IgG antibody. In all cases, the qualitative outcome of the positive sample remained unchanged. The negative sample showed a range of recovery from 1520.8% with the high spike of IgG to a low of 66.7% with the high spike of hemoglobin. With the exception of the spiking of purified human IgG, the qualitative outcome of the sample was unaffected with these substances. Finally, the borderline sample showed a range of recovery from 352% with the high spike of purified human IgG to a low of 93.1% for the high spike of hemoglobin. It can be concluded that all substances tested showed some level of interference with detection of anti-Measles antibody in the **AtheNA Multi-Lyte** MMV IgG Plus Test System 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 **AtheNA Multi-Lyte** MMV IgG Plus Test System.

2. **Mumps Analyte**

a. **A comparative study** was conducted where a total of 253 specimens were tested. Of the 253 specimens tested, 177 were prospective specimens and 76 were retrospective specimens. The prospective specimens were tested at a centralized hospital laboratory located in Southeastern US and in a reference laboratory located in the Midwest. See the Expected Results section for the demographic distribution of these samples. The retrospective specimens were comprised of 76 pregnant women ranging in age from 18 to 41. Of these 76 pregnant women, 16/76 were in their first trimester of pregnancy, 30/76 were in their second trimester and 30/76 were in their third trimester of pregnancy. Specimens were tested using the AtheNA Multi-Lyte® MMV IgG Plus Test System and a reference Mumps IgG test system (either the ZEUS ELISA Mumps IgG Test System or the Bion Mumps IgG IFA test system. The results of this comparative study are depicted below in Tables 10 - 14:

Table 10: Site One Prospective Samples

		ZEUS ELISA Mumps IgG Test System Results			
		Positive	Negative	Equivocal*	Total
AtheNA Multi-Lyte MMV IgG Plus Test System	Positive	84	0	0	84
	Negative	0	0	0	0
	Equivocal*	3	0	0	3
	Invalid	0	0	0	0
	Total	87	0	0	87
Positive % Agreement = 84/87 = 96.6%			95% Confidence Interval – 92.7 to 100.4%		
Negative % Agreement = 0/0 = N/A			95% Confidence Interval – N/A		
Overall Agreement = 84/87 = 96.6%			95% Confidence Interval – 92.7 to 100.4%		
*AtheNA specimens showing equivocal results were considered to be as “non-agreement” specimens.					

Table 11: Site Two Prospective Samples

		Bion Mumps IgG IFA Results			
		Positive	Negative	Equivocal*	Total
AtheNA Multi-Lyte MMV IgG Plus Test System	Positive	58	0	0	58
	Negative	15	9	0	24
	Equivocal*	9	0	0	9
	Invalid**	0	1	0	1
	Total	82	10	0	92
Positive % Agreement = 58/82 = 70.7%			95% Confidence Interval – 60.9 to 80.6%		
Negative % Agreement = 9/9 = 100.0%			95% Confidence Interval – 100.0 to 100.0%		
Overall Agreement = 67/91 = 73.6%			95% Confidence Interval –64.6 to 82.7%		
*AtheNA specimens showing equivocal results were considered to be as “non-agreement” specimens.					
**Specimens showing invalid results were excluded from calculations for agreement.					

Table 12: Combined Sites Prospective Samples

		Reference Testing Results			
		Positive	Negative	Equivocal*	Total
AtheNA Multi-Lyte MMV IgG Plus Test System	Positive	140	0	0	140
	Negative	15	9	0	24
	Equivocal*	12	0	0	12
	Invalid**	0	1	0	1
	Total	167	10	0	177
Positive % Agreement = 140/167 = 83.8%			95% Confidence Interval – 78.2 to 89.4%		
Negative % Agreement = 9/9 = 100.0%			95% Confidence Interval – 100.0 to 100.0%		
Overall Agreement = 149/176 = 84.7%			95% Confidence Interval –79.3 to 90.0%		
*AtheNA specimens showing equivocal results were considered to be as “non-agreement” specimens.					
**Specimens showing invalid results were excluded from calculations for agreement.					

Table 13: Retrospective Samples

		Bion Mumps IgG IFA Results			
		Positive	Negative	Equivocal	Total
AtheNA Multi-Lyte MMV IgG Plus Test System	Positive	60	1	0	71
	Negative	0	12	0	5
	Equivocal	0	2	1	0
	Invalid	0	0	0	0
	Total	60	15	0	76
Positive % Agreement = 60/76 = 78.9%			95% Confidence Interval – 69.8 to 88.1%		
Negative % Agreement = 12/15 = 80.0%			95% Confidence Interval – 59.8 to 100.2%		
Overall Agreement = 72/76 = 94.7%			95% Confidence Interval – 89.7 to 99.8%		
*AtheNA and IFA specimens showing equivocal results were considered to be as “non-agreement” specimens.					

Table 14: All Samples Combined

		Reference Testing Results			
		Positive	Negative	Equivocal*	Total
AtheNA Multi-Lyte MMV IgG Plus Test System	Positive	200	1	0	201
	Negative	15	21	0	36
	Equivocal*	12	2	1	15
	Invalid**	0	1	0	1
	Total	227	25	1	253
Positive % Agreement = 200/227 = 88.1%		95% Confidence Interval – 83.9 to 92.3%			
Negative % Agreement = 21/24 = 87.5%		95% Confidence Interval – 74.3 to 100.7%			
Overall Agreement = 221/252 = 87.7%		95% Confidence Interval –83.8 to 91.8%			
*AtheNA, ELISA and IFA specimens showing equivocal results were considered to be as "non-agreement" specimens.					
**Specimens showing invalid results were excluded from calculations for agreement.					

b. **A precision study** was conducted at multiple sites as follows: Six samples were identified for use in the study based upon their activity on the **AtheNA Multi-Lyte** MMV IgG 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 the 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. The summary of the precision study appears below in Table 15.

Table 15: Mumps Precision

		Intra-Assay									Inter-Assay			Between Sites
		Site One			Site Two			Site Three						
Sample		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Site One	Site Two	Site Three	
1	Mean	287.4	233.1	253.6	258.4	280.5	259.5	287.4	277.8	282.1	258.0	266.1	282.4	268.9
	StD	14.3	12.0	12.9	15.0	19.5	14.1	14.3	20.8	13.6	26.1	18.8	16.3	22.9
	% CV	5.0	5.1	5.1	5.8	7.0	5.4	5.0	7.5	4.8	10.1	7.1	5.8	8.5
2	Mean	361.4	314.8	315.9	351.6	336.0	305.1	361.4	373.1	357.5	330.7	330.9	364.0	341.9
	StD	11.7	16.1	23.8	11.1	18.1	21.6	11.7	22.9	10.8	28.0	25.9	16.8	38.5
	% CV	3.2	5.1	7.5	3.2	5.4	7.1	3.2	6.1	3.0	8.5	7.8	4.6	8.3
3	Mean	55.9	33.8	34.3	39.5	41.3	32.4	55.9	53.0	57.3	41.3	37.7	55.4	44.8
	StD	5.1	2.3	2.8	3.0	4.8	7.9	5.1	3.4	3.5	11.1	6.6	4.3	10.9
	% CV	9.1	6.9	8.1	7.7	11.6	24.4	9.1	6.4	6.1	26.8	17.6	7.8	24.4
4	Mean	64.9	30.9	40.4	40.8	46.0	35.1	64.9	60.0	63.4	45.4	40.6	62.8	49.6
	StD	3.6	6.6	6.7	3.0	4.7	2.4	3.6	6.1	3.6	15.6	5.6	4.9	13.7
	% CV	5.6	21.2	16.6	7.4	10.3	6.9	5.6	10.2	5.7	34.5	13.9	7.8	27.7
5	Mean	183.4	151.6	138.0	143.8	163.3	150.6	183.4	179.6	184.1	157.7	152.5	182.4	164.2
	StD	8.6	9.5	8.3	15.9	8.6	14.8	8.6	7.9	5.1	21.2	15.3	7.3	20.3
	% CV	4.7	6.2	6.1	11.0	5.3	9.8	4.7	4.4	2.7	13.4	10.0	4.0	12.3
6	Mean	128.5	94.3	88.1	116.9	117.1	92.3	128.5	125.6	138.3	103.6	108.8	130.8	114.4
	StD	5.9	8.0	15.9	6.5	3.9	8.5	5.9	13.3	9.9	20.9	13.5	11.2	19.5
	% CV	4.6	8.5	18.0	5.6	3.3	9.2	4.6	10.6	7.2	20.1	12.4	8.5	17.1

c. **A cross reactivity study** was conducted where 10 serum specimens were selected that were negative on the **AtheNA Multi-Lyte** MMV IgG Plus Test System and were subsequently tested by commercially available ELISAs for IgG antibody activity to HSV-1 & 2, CMV, EBV Nuclear Antigen, EBV EA, EBV Viral Capsid Antigen and Toxoplasma. Nine of the ten samples were positive for one or more of the viral markers tested. The results of this study appear below in Table 16.

Table 16: Mumps Cross Reactivity

	AtheNA Multi-Lyte	ELISA Results					
Sample	**Results**	CMV	HSV-1	HSV-2	EBNA	EBV-EA IgG	EBV
CN 18	27	0.10	5.46	1.22	6.44	0.18	5.89
CN 35	70	7.96	6.96	4.69	8.75	0.50	4.79
CN 70	77	0.28	7.45	2.42	8.25	0.25	4.78
CN 77	37	2.82	5.26	2.94	1.95	0.24	3.82
CN 122	54	1.30	0.25	0.13	0.00	0.72	0.70
CN 140	27	1.88	1.06	0.16	3.14	0.20	0.82
CN 141	93	2.41	3.08	0.62	5.46	0.24	2.97
CN 144	35	1.75	0.87	0.14	2.00	0.21	0.92
CN 146	31	1.92	1.31	0.36	3.83	0.84	1.42
CN 149	77	1.52	0.38	0.22	6.58	0.39	1.33

d. **An interfering substances** study was conducted to determine the potential effects of interfering substances that may be found in serum specimens. The following potentially interfering substances were spiked into serum specimens at the levels indicated:

Table 17: Mumps Interfering Substances Levels

Substance	Low Spike	High Spike
Bilirubin	1.9mg/dL	3.8mg/dL
Human Albumin	5.5g/dL	11g/dL
Human IgG	1.8g/dL	3.6g/dL
Cholesterol	200mg/dL	400mg/dL
Triglycerides	150mg/dL	300mg/dL
Hemoglobin	180g/dL	360g/dL
Intralipids	3.5mg/mL	7.0mg/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, three sera were evaluated in the presence of each of the substances above. One specimen was clearly postive for Mumps IgG, one was borderline and one was clearly negative for Mumps IgG. The results of the control specimens and the low and high spiked sera are presented in the Table 18 below.

Table 18: Mumps Interfering Substance		Sample One		Sample Two		Sample 3	
Interfering Substance	**Spike Level**	**Mumps Positive**	**% Positive Signal Recovered**	**Mumps Equivocal**	**% Positive Signal Recovered**	**Mumps Negative**	**% Positive Signal Recovered**
None (Control)	N/A	245	N/A	89	N/A	60	N/A
Bilirubin	Low	276	112.7	123	138.2	59	98.3
Bilirubin	High	253	103.3	94	105.6	60	100.0
Albumin	Low	261	106.5	97	109.0	61	101.7
Albumin	High	249	101.6	89	100.0	55	91.7
IgG	Low	376	153.5	379	425.8	367	611.7
IgG	High	415	169.4	388	436.0	437	728.3
Cholesterol	Low	264	107.8	97	109.0	63	105.0
Cholesterol	High	2268	109.4	103	115.7	68	113.3
Triglycerides	Low	253	103.3	102	114.6	58	96.7
Triglycerides	High	248	101.2	94	105.6	60	100.0
Hemoglobin	Low	231	94.3	99	111.2	60	100.0
Hemoglobin	High	229	93.5	95	106.7	52	86.7
Intralipid	Low	250	102.0	103	115.7	68	113.3
Intralipid	High	379	154.7	103	115.7	59	98.3

The positive sample showed a range of recovery from 169.4% with the high spike of IgG to a low of 93.5% with the high spike of hemoglobin. The addition of purified IgG caused a significant rise in signal since it is likely the purified human IgG used to spike the specimen was positive for anti-Mumps IgG antibody. In all cases, the qualitative outcome of the positive sample remained unchanged. The negative sample showed a range of recovery from 728.3% with the high spike of IgG to a low of 91.7% with the high spike of albumin. With the exception of the spiking of purified human IgG, the qualitative outcome of the sample was unaffected with these substances. Finally, the borderline sample showed a range of recovery from 436% with the high spike of purified human IgG to a low of 100% for the high spike of albumin. It can be concluded that all substances tested showed some level of interference with detection of anti-Mumps antibody in the **AtheNA Multi-Lyte** MMV IgG Plus Test System 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 **AtheNA Multi-Lyte** MMV IgG Plus Test System.

3. **VZV Analyte**

a. **A comparative study** was conducted where there were a total of 272 specimens tested. Of the 272 specimens tested, 177 were prospective specimens and 95 were retrospective specimens. The prospective specimens were tested at a centralized hospital laboratory located in Southeastern US and in a reference laboratory located in the Midwest. See the Expected Results section for the demographic distribution of these samples. The 95 retrospective specimens were comprised of 19 children ranging in age from one to 12 years and 76 pregnant women ranging in age from 18 to 41. Of these 76 pregnant women, 16/76 were in their first trimester of pregnancy, 30/76 were in their second trimester and 30/76 were in their third trimester of pregnancy. Specimens were tested using the **AtheNA Multi-Lyte** MMV IgG Plus Test System and the ZEUS ELISA VZV IgG Test System. The results of this comparative study are depicted below in Table 19 - 22.

Table 19: Site One Prospective Samples		ZEUS ELISA VZV IgG Test System Results			
		Positive	Negative	Equivocal*	Total
AtheNA Multi-Lyte MMV IgG Plus Test System	Positive	83	0	0	83
	Negative	1	0	0	1
	Equivocal*	3	0	0	3
	Invalid	0	0	0	0
	Total	87	0	0	87
Positive % Agreement = 83/87 = 95.4%		95% Confidence Interval – 88.6 to 98.7%			
Negative % Agreement = 0/0 = N/A		95% Confidence Interval – N/A			
Overall Agreement = 83/87 = 95.4%		95% Confidence Interval – 88.6 to 98.7%			
*AtheNA specimens showing equivocal results were considered to be as "non-agreement" specimens.					

Table 20: Site Two Prospective Samples		ZEUS ELISA VZV IgG Test System Results			
		Positive	Negative	Equivocal*	Total
AtheNA Multi-Lyte MMV IgG Plus Test System	Positive	61	0	2	63
	Negative	5	16	3	24
	Equivocal*	2	0	0	2
	Invalid**	0	1	0	1
	Total	68	17	5	90
Positive % Agreement = 61/71 = 85.9%		95% Confidence Interval – 75.6 to 93.0%			
Negative % Agreement = 16/18 = 88.9%		95% Confidence Interval – 65.3 to 98.6%			
Overall Agreement = 77/89 = 86.5%		95% Confidence Interval –77.6 to 92.8%			
*AtheNA and ELISA specimens showing equivocal results were considered to be as "non-agreement" specimens.					
**Specimens showing invalid results were excluded from calculations for agreement.					

Table 21: Combined Sites Prospective Samples		ZEUS ELISA VZV IgG Test System Results			
		Positive	Negative	Equivocal*	Total
AtheNA Multi-Lyte MMV IgG Plus Test System	Positive	144	0	2	146
	Negative	6	16	3	25
	Equivocal*	5	0	0	5
	Invalid**	0	1	0	1
	Total	155	17	5	177
Positive % Agreement = 140/158 = 91.9%		95% Confidence Interval – 85.6 to 95.1%			
Negative % Agreement = 16/18 = 88.9%		95% Confidence Interval – 65.3 to 98.6%			
Overall Agreement = 160/176 = 90.9%		95% Confidence Interval –85.7 to 94.7%			
*AtheNA and ELISA specimens showing equivocal results were considered to be as "non-agreement" specimens.					
**Specimens showing invalid results were excluded from calculations for agreement.					

Table 22: Retrospective Samples

		ZEUS ELISA VZV IgG Test System Results			
		Positive	Negative	Equivocal	Total
AtheNA Multi-Lyte MMV IgG Plus Test System	Positive	72	0	0	72
	Negative	5	12	2	19
	Equivocal	3	0	0	3
	Invalid	1	0	0	1
	Total	81	12	2	95
Positive % Agreement = 72/80 = 90.0%		95% Confidence Interval – 83.4 to 96.6%			
Negative % Agreement = 12/12 = 100.0%		95% Confidence Interval – 73.5 to 100.0%			
Overall Agreement = 84/94 = 89.4%		95% Confidence Interval – 81.3 to 94.8%			
*AtheNA and ELISA specimens showing equivocal results were considered to be as "non-agreement" specimens.					
**Specimens showing invalid results were excluded from calculations for agreement.					

b. **Assay precision** was evaluated at multiple sites as follows: six samples were identified for use in the study based upon their activity on the AtheNA assay. 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 the 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. The summary of the precision study appears below in Table 23.

Table 23: VZV Precision

		Intra-Assay									**Inter-Assay**			**Between Sites**
		Site One			Site Two			Site Three						
Sample		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Site One	Site Two	Site Three	
1	Mean		318			311			334		318	311	334	321
	StD	15.48	16.69	24.48	17.20	16.53	13.29	16.81	23.42	10.94	27.75	20.22	17.57	23.96
	% CV	4.9	4.8	8.3	5.5	5.6	4.3	5.0	7.1	3.3	8.7	6.5	5.3	7.5
2	Mean		264			262			290		264	262	290	272
	StD	15.59	8.87	9.65	14.66	21.63	22.41	19.04	26.10	15.33	14.38	19.59	21.06	22.29
	% CV	5.9	3.2	3.8	5.5	8.3	8.7	6.5	8.8	5.5	5.4	7.5	7.3	8.2
3	Mean		25			25			35		25	25	35	28
	StD	1.64	2.07	2.98	3.11	2.56	2.43	1.41	2.07	1.93	3.14	3.06	1.85	5.53
	% CV	6.5	7.5	13.5	11.4	11.0	9.5	4.0	6.0	5.4	12.7	12.0	5.3	19.4
4	Mean		13			11			21		13	11	21	15
	StD	1.77	2.53	0.99	2.23	1.55	1.07	1.69	1.28	0.74	2.46	2.65	1.37	4.74
	% CV	14.3	17.0	9.1	15.8	17.0	9.7	7.8	6.3	3.6	19.3	23.2	6.6	31.6
5	Mean		105			102			101		105	102	101	103
	StD	5.36	2.88	12.98	4.29	4.53	4.61	2.83	8.27	8.55	10.41	7.95	6.93	8.60
	% CV	5.3	2.5	12.9	4.7	4.5	4.8	2.8	8.3	8.3	9.9	7.8	6.8	8.4
6	Mean		88			89			128		88	89	128	102
	StD	3.81	4.33	7.86	15.63	4.54	19.40	6.63	6.10	7.03	9.20	14.22	6.37	21.36
	% CV	4.3	4.5	10.00	17.3	5.3	21.5	5.2	4.7	5.6	10.5	16.0	5.0	21.0

c. **Two separate cross reactivity studies** were conducted to assess potential cross reactivity to other viruses. In the first study, 15 serum specimens were selected that were negative on the **AtheNA Multi-Lyte** MMV IgG Plus Test System and were subsequently tested by commercially available ELISAs for activity to Measles, Mumps and Rubella. Of the 15 samples, two were negative on all three ELISA tests, but 13/15 were IgG positive for one or more of the three viruses. In the second study, 16 serum samples were selected that were negative for VZV IgG antibody by both a commercially available ELISA and AtheNA. These samples were subsequently tested by commercially available ELISAs for IgG antibody to HSV-1, CMV, Hepatitis B, EBV nuclear antigen, and EBV viral capsid antigen. All 16 samples were positive for one or more of the viral markers tested. The results of these two studies appear below in Tables 24 and 25:

Table 24: VZV Cross Reactivity Study One

	AtheNA Multi-Lyte Results	**ELISA Results**		
Sample		Measles IgG	Mumps IgG	Rubella IgG
1	Negative	Positive	Positive	Positive
2	Negative	Positive	Positive	Positive
3	Negative	Positive	Positive	Positive
4	Negative	Positive	Negative	Negative
5	Negative	Positive	Positive	Positive
6	Negative	Positive	Positive	Positive
7	Negative	Positive	Positive	Positive
8	Negative	Positive	Negative	Positive
9	Negative	Positive	Positive	Positive
10	Negative	Positive	Positive	Positive
11	Negative	Negative	Negative	Negative
12	Negative	Negative	Negative	Positive
13	Negative	Positive	Negative	Negative
14	Negative	Negative	Negative	Positive
15	Negative	Negative	Negative	Negative

Table 25: VZV Cross Reactivity Study Two

Sample	AtheNA Multi-Lyte Results	ELISA Results					
		EBNA	EBV-VCA	CMV	HSV-1	HSV-2	Hepatitis Ab
1	Negative	Not Tested	Positive	Positive	Positive	Negative	Negative
2	Negative	Positive	Positive	Positive	Positive	Positive	Positive
3	Negative	Positive	Positive	Negative	Positive	Negative	Negative
4	Negative	Not Tested	Positive	Negative	Negative	Negative	Negative
5	Negative	Positive	Positive	Negative	Positive	Equivocal	Negative
6	Negative	Positive	Positive	Negative	Positive	Negative	Negative
7	Negative	Negative	Equivocal	Positive	Positive	Positive	Negative
8	Negative	Positive	Positive	Negative	Positive	Equivocal	Negative
9	Negative	Positive	Positive	Negative	Positive	Negative	Negative
10	Negative	Positive	Positive	Negative	Negative	Negative	Negative
11	Negative	Not Tested	Positive	Positive	Equivocal	Negative	Negative
12	Negative	Not Tested	Positive	Positive	Equivocal	Negative	Negative
13	Negative	Positive	Positive	Positive	Negative	Negative	Negative
14	Negative	Positive	Positive	Positive	Positive	Negative	Positive
15	Negative	Positive	Positive	Positive	Negative	Negative	Negative
16	Negative	Positive	Positive	Negative	Positive	Negative	Positive

d. **An interfering substance study** was conducted to determine the potential effects of interfering substances that may be found in serum specimens. Table 26 outlines the levels of potentially interfering substances that were spiked into serum specimens. 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.

Table 26: VZV Interfering Substances Levels

Substance	Low Spike	High Spike
Bilirubin	1.9mg/dL	3.8mg/dL
Human Albumin	5.5g/dL	11g/dL
Human IgG	1.8g/dL	3.6g/dL
Cholesterol	200mg/dL	400mg/dL
Triglycerides	150mg/dL	300mg/dL
Hemoglobin	180g/dL	360g/dL
Intralipids	3.5mg/mL	7.0mg/mL

For this study, three sera were evaluated in the presence of each of the substances above. One specimen was clearly postive for VZV IgG, one was borderline and one was clearly negative for VZV IgG. The results of the control specimens and the low and high spiked sera are presented in the Table 27.

Table 27: VZV Interfering Substances

Interfering Substance	Spike Level	Sample One		Sample Two		Sample 3	
		VZV IgG Positive	% Positive Signal Recovered	VZV IgG Equivocal	% Positive Signal Recovered	VZV IgG Negative	% Positive Signal Recovered
None (Control)	N/A	177	N/A	114	N/A	25	N/A
Bilirubin	Low	155	87.6	117	102.6	50	200.0
Bilirubin	High	127	71.8	95	83.3	48	192.0
Albumin	Low	163	92.1	111	97.4	55	220.0
Albumin	High	126	71.2	1117	102.6	62	248.0
IgG	Low	300	169.5	295	258.8	344	1376.0
IgG	High	446	252.0	313	274.6	452	1808.0
Cholesterol	Low	147	83.1	113	99.1	58	232.0
Cholesterol	High	164	92.7	115	100.9	57	228.0
Triglycerides	Low	137	77.4	99	86.8	52	208.0
Triglycerides	High	142	80.2	88	77.2	46	184.0
Hemoglobin	Low	129	78.0	101	88.6	51	204.0
Hemoglobin	High	129	72.9	113	99.1	44	176.0
Intralipid	Low	139	78.5	101	88.6	56	224.0
Intralipid	High	163	92.1	99	86.8	51	244.0

The positive sample showed a range of recovery from 252% with the high spike of IgG to a low of 71.2% with the high spike of albumin. The addition of purified IgG caused a significant rise in signal since it is likely the purified human IgG used to spike the specimen was positive for anti-VZV IgG antibody. In all cases, the qualitative outcome of the positive sample remained unchanged. The negative sample showed a range of recovery from 1808% with the high spike of IgG to a low of 176% with the high spike of hemoglobin. With the exception of the spiking of purified human IgG, the qualitative outcome of the sample was unaffected with these substances. Finally, the borderline sample showed a range of recovery from 274.5% with the high spike of purified human IgG to a low of 83.3% for the high spike of bilirubin. It can be concluded that all substances tested showed some level of interference with detection of anti-VZV antibody in the AtheNA Plus assay 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 **AtheNA Multi-Lyte** MMV IgG Plus Test System.

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