ToRCH IgG Plus Test System

REF A81101G

IVD

Rx Only

INTENDED USE

The ZEUS AtheNA Multi-Lyte® ToRCH IgG Plus Test System is intended intended for the qualitative detection of specific human IgG class antibodies to Toxoplasma gondii (T.gondii), Rubella, Cytomegalovirus (CMV), HSV-1 and HSV-2 in human serum. The results of this assay are intended to be used as an aid in the assessment of serological status to Toxoplasma gondii, Rubella and CMV. For HSV-1 and HSV-2, the test is indicated for sexually active adults and expectant mothers, as an aid for presumptively diagnosing Herpes Simplex 1 and Herpes Simplex 2. The test is not intended for use in screening blood or plasma donors. The performance of this assay has not been established for use in a pediatric population, neonatal screening, immunocompromised or immunosuppressed patients or for use at point of care facilities. This test is for In Vitro diagnostic use only.

SIGNIFICANCE AND BACKGROUND

T. gondii is an obligate intracellular protozoan parasite with a worldwide distribution (1, 2). Infection with T. gondii is asymptomatic in the majority (80-90%) of cases (3). The most common clinical manifestation of acute toxoplasmosis in the adult is asymptomatic lymphadenopathy involving single or multiple nodes. Lymphadenopathy may be accompanied by fever, malaise, and atypical lymphocytosis symptoms that mimic infectious mononucleosis. Very rarely will more serious complications, such as encephalitis, myocarditis or pneumonitis, be seen in the normal host (1). During infection of a seronegative woman with T. gondii during pregnancy, transmission of the organism occurs across the placenta to the fetus (1, 4). The severity of infection in the fetus varies with the trimester during which the acquisition of the infection occurred. Infection during the first trimester may lead to spontaneous abortion, stillbirth, or overt disease in the neonate. Infection acquired later during pregnancy is usually asymptomatic in the neonate, and may not be recognized (4). Varieties of serologic tests for antibodies to T. gondii have been used as an aid in diagnosis of acute infection, and to assess previous exposure to the organism. The more widely used tests include the Sabin-Feldman dye test, direct agglutination, indirect hemagglutination, latex agglutination, indirect immunofluorescence, ELISA (5) and PCR (6).

Rubella is a mild, contagious viral infection that occurs primarily in children and young adults (7, 8). An erythematous maculopapular rash that lasts two or three days characterizes rubella. However, greater than 50% of rubella infections are not clinically apparent (8). Other symptoms of rubella may include low-grade fever, mild upper respiratory symptoms, and suboccipital lymphadenopathy. Transient arthralgia and arthritis are common symptoms in young adults but more severe complications such as encephalitis or thrombocytopenic purpura are uncommon (7). Although rubella infection is usually self-limiting, infection of the fetus during the first trimester may cause spontaneous abortion, stillbirth or congenital birth defects (9). Congenital rubella syndrome has long been recognized and is characterized by congenital heart disease, cataracts, neurosensory deafness, mental retardation, and intrauterine growth retardation (7, 11). Following an epidemic of rubella in 1964, new clinical manifestations of congenital rubella were recognized. They included neonatal thrombocytopenic purpura, hepatitis, bone lesions and meningoencephalitis (10). In addition, diabetes mellitus and progressive rubella panencephalitis are late-emerging manifestations of congenital rubella infection that have recently been recognized (7). Rubella is endemic worldwide (8). In countries without vaccination programs, 10-25% of women of childbearing age are seronegative and susceptible to infection (8). Extensive vaccination programs in the United States and the United Kingdom have greatly reduced the incidence of congenital rubella syndrome (7, 12). Currently, reports show fewer than 10 cases per year in the United States. The presence of circulating maternal antibody indicates immunity to rubella (7, 12, and 13).

Cytomegalovirus (CMV) infections are widespread and usually asymptomatic; however, the virus may persist as a latent or chronic infection (14). The relatively frequent incidence and often-severe disease in newborns and immunosuppressed individuals clearly establishes this agent as an important human pathogen (15 - 17). CMV infections can be classified as follows:

Congenital - Acquired before birth
Perinatal - Acquired at birth
Postnatal - Acquired after birth

Acquisition of postnatal CMV infections occurs through close contact with individuals who are shedding the virus (15). CMV has been isolated from saliva, urine, breast milk, cervical secretions, and semen. Consequently, the transmission of the virus may occur through a variety of mechanisms (18 - 20). Sexual transmission of the virus appears to contribute to the acquisition of the virus by young adults (21). Although the age at which CMV infection is acquired varies with socioeconomic conditions, only about 10 - 15% of children in the United States are seropositive. By age 35 however, about 50% of the population is seropositive (15 - 17). The majority of individuals contracting postnatal CMV infections remain asymptomatic (15 - 17). A small percentage of individuals will develop a negative heterophile-antibody infectious mononucleosis syndrome. Characteristics of CMV mononucleosis are fever, lethargy, and atypical lymphocytosis; whereas, in Epstein-Barr virus induced infectious mononucleosis, pharyngitis, lymphadenopathy, and splenomegaly are the chief clinical features (22, 23). Serologic procedures, which measure IgG antibodies to CMV, can aid in the diagnosis of CMV infection when seroconversion can be demonstrated (24).

Herpes Simplex Virus (HSV) infections are caused by two distinct types of HSV; HSV-1 and HSV-2. Both HSV types are common human pathogens. HSV-1 is usually associated with infections in the oropharyngeal area and eyes while HSV-2 causes most genital infections (25, 26). However, HSV-2 can be isolated occasionally from the oropharyngeal area (27) and 15 to 20% of primary genital infections may be caused by HSV-1 (25, 27). HSV infections are transmitted by virus-containing secretions through close personal contact. HSV infections, both primary and recurrent are often subclinical and asymptomatic. Shedding of the virus is the most important factor contributing to the spread of the virus (26). The most severe complication of genital HSV infection is neonatal disease (26). Of mothers with an active primary infection, the risk of transmission to infants is as high as 40% (28). About 69 - 80% of infants who develop neonatal herpes are born to women who are asymptomatic of genital HSV infection at the time of birth (28). Genital herpes is problematic in sexually active adults as well as the disease is often transmitted in the absence of symptoms (33). HSV antibody testing is indicated for sexually active adults to identify those at risk for acquiring HSV or transmitting HSV to others and for expectant mothers who are at risk for acquiring HSV infections and transmitting neonatal herpes (29, 33). Although culture combined with direct fluorescent antibody (DFA) testing is definitive in making a diagnosis, the timing is critical and cultures must be obtained during periods of active disease to produce optimal recovery (30, 31). Serological procedures may be useful for determining evidence of infection with HSV (32). Many existing serologic methods for determining HSV sero-status, however, are unable to differentiate between HSV-1 and HSV-2 infections (32). Development of HSV type-specific serological assays occurred using the significant difference between the gG-1 protein of HSV-1 and the gG-2 protein of HSV-2 (32). There are benefits to early application of type-specific serologic testing for HSV-1 and HSV-2 in testing first time, recurrent, and asymptomatic infections to aid in diagnosis and appropriate patient counseling (33). Type-specific testing is recommended for sexually active adults and pregnant women (34).

PRINCIPLE OF THE ASSAY

The ZEUS **AtheNA Multi-Lyte** ToRCH IgG Plus Test System is a multiplexed assay designed to detect IgG antibodies specific for *Toxoplasma gondii*, Rubella, CMV, HSV-1 gG-1 and HSV-2 gG-2 in human sera. The test procedure involves two incubation steps:

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

3. Positive Control 1 (Human Serum): One, red-capped vial containing 0.2mL.

CONTROL	+	2

4. Positive Control 2 (Human Serum): One, white-capped vial containing 0.2mL.

CONTROL	-

5. Negative Control (Human Serum): One, green-capped vial containing 0.2mL..

DIL	SPE

6. 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

7. 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 (36).
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 Controls, 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 (13, 14). 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 (40).

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 Control and the two Positive Controls with each run. The Negative Control should be tested in well A1, Positive Control 1 in well B1 and Positive Control 2 in well C1. 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 1	
C	Positive Control 2	
D	Patient 1	
E	Patient 2	
F	Patient 3	
G	Patient 4	
H	Patient 5	

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of SAVe Diluent®) of the Negative Control, Positive Controls, 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 ToRCH 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 two Positive Controls (in wells B1 through C1).
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 Control and the two 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. Each Positive Control must be positive for a predetermined group of analytes included in the Bead Suspension. These ranges are encoded within the Calibration CD. Positive Control ranges may be viewed by clicking on the "Control Graphs" button of the AtheNA software and then clicking "Control Upper/Lower Limits."
   d. **Failure to meet any of the above criteria must result in non-reporting of patient results**. Consider the entire run invalid and repeat.
3. Specimen validity is based upon the characteristics of the calibration beads and their interactions with the patient sera. There are various parameters monitored automatically through *Intra-Well Calibration Technology*. If any of the criteria are found to be out of specification, the patient's results are considered invalid and should be repeated. Should this occur, the data report will indicate the particular specimen which has been invalidated as well as a trouble shooting code.
4. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. External Controls must be representative of normal human serum since **AtheNA Multi-Lyte's** calibration system is partially based upon the characteristics of the serum sample. If the specimen formulation is artificial (not human serum), erroneous results may occur.
5. Good laboratory practice recommends the use of Positive and Negative Controls to assure functionality of reagents and proper performance of the assay procedure. Quality control requirements must be performed in conformance with local, state and/or federal regulations or accreditation requirements and your laboratory' s standard Quality Control procedures. ZEUS recommends 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** ToRCH 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** ToRCH 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. **Result Interpretation For Toxoplasma, CMV, HSV-1 and HSV-2:**

<100 AU/mL: Interpret result as negative. No significant amount of IgG antibody to Toxoplasma, CMV, HSV-1 or HSV-2 IgG detected.
>120 AU/mL: Interpret result as positive. IgG antibodies to Toxoplasma, CMV, HSV-1 or HSV-2 detected.
100 - 120 AU/mL: Interpret result as equivocal for presence of IgG antibody to Toxoplasma, CMV, HSV-1 or HSV-2. Re-test equivocal samples in duplicate. If upon re-testing, one of the two samples remains equivocal, test the sample by an alternate serological procedure such as ELISA or draw a second sample one to three weeks later and test.
INV NSC: Indicates too much activity on the non-specific control bead. Re-test the sample, if result remains invalid, re-evaluate with a fresh sample.

   b. **Result Interpretation for Rubella**:

0 - 9 Interpret result as negative. Detection of IgG antibodies for Rubella did not occur. Presume that patient is not immune against infection with Rubella.
11 or Greater: Interpret result as positive. IgG antibodies to Rubella were detected at a level that indicates immunity to infection.
10: Interpret result as indeterminate for presence of IgG antibody to Rubella virus. Re-test indeterminate samples in duplicate. If upon re-testing one of the two samples remain equivocal, test the sample by an alternate serological procedure such as ELISA, or draw a second sample one to three weeks later and test.
INV NSC: Indicates too much activity on the non-specific control bead. Re-test the sample, if result remains invalid, re-evaluate with a fresh sample.

   NOTE: ZEUS Scientific technicians calibrated this test to the first WHO International Standard for Rubella IgG at the cut-off. The result of 11 is equivalent to the cutoff of 10 IU/ml of the WHO Standard for Rubella. The magnitude of the test result above or below the cut-off does not correspond to International Units and is not indicative of total amount of antibody present.

LIMITATIONS OF THE ASSAY

1. Performance characteristics of this device have not been established for matrices other than serum.
2. Samples collected too early in the course of the infection may not have detectable levels of IgG antibodies. Negative results may be due to delayed seroconversion.
3. The numeric value of the result above the cutoff is not indicative of the amount of anti-Toxoplasma, Rubella, CMV, HSV-1 or HSV-2 IgG antibody present.
4. False positive results may occur. Repeat testing, or testing with a different device may be necessary in some settings e.g. symptomatic patients.
5. The ZEUS **AtheNA Multi-Lyte** ToRCH IgG Plus Test System is a diagnostic aid and by itself is not diagnostic. Test results should be interpreted in conjunction with the clinical history, epidemiological data and other information available to the attending physician in evaluating the patient and the results of other diagnostic procedures.
6. Hemolytic, icteric, or lipemic samples may interfere with the outcome of this assay. Additionally, specimens with abnormal IgG concentrations may interfere with the outcome of this assay. Use of these types of specimens should be avoided.

EXPECTED RESULTS

ZEUS Scientific technicians evaluated the observed prevalence at three sites in a prospective study including individuals and pregnant women undergoing ToRCH testing.

1. Six hundred and fifty-one masked samples prospectively collected from individuals between the ages of <1 and 89 were tested at two external sites. Three hundred samples were submitted for ToRCH antibody assessment and 351 samples were submitted for testing of one or more of the analytes in the ToRCH panel. Technicians performed testing for all five markers on all samples. Results from a subset of 596/651 individuals between the ages of 17 and 69 were used to calculate HSV-1 and HSV-2 prevalence. Site One, a hospital laboratory located in the Mid-Atlantic region tested 300 samples. Site Two, a hospital laboratory in the Northeast tested 351 samples.
2. Two hundred masked samples prospectively collected from pregnant women for ToRCH antibody assessment were obtained from two serum vendors. The women ranged in age from 15 to 46. Testing of the samples for all 5 analytes occurred internally.

Table 1: Observed Prevalence in Individuals Undergoing ToRCH Antibody Assessment

Prevalence of Analytes in Prospective Samples							
		Toxoplasma		Rubella		CMV	
Age	Gender	Pos/Total	% Prevalence	Pos/Total	% Prevalence	Pos/Total	% Prevalence
0-9	Male	0/2	0.0	1/2	50.0	1/2	50.0
	Female	1/2	50.0	1/2	50.0	1/2	50.0
10-19	Male	1/3	33.3	3/3	100.0	2/3	66.7
	Female	5/55	9.1	50/55	90.9	38/55	69.1
20-29	Male	2/24	8.3	23/24	95.8	9/24	37.5
	Female	58/257	22.6	232/257	90.3	184/257	71.6
30-39	Male	3/16	18.8	12/16	75.0	8/16	50.0
	Female	57/189	30.2	170/189	89.9	145/189	76.7
40-49	Male	6/11	54.5	11/11	100.0	8/11	72.7
	Female	14/44	31.8	39/44	88.6	30/44	68.2
50-59	Male	1/10	10.0	9/10	90.0	5/10	50.0
	Female	3/11	27.3	10/11	90.9	10/11	90.9
60-69	Male	3/6	50.0	6/6	100.0	4/6	66.7
	Female	3/4	75.0	4/4	100.0	3/4	75.0
70+	Male	0/0	0.0	0/0	0.0	0/0	0.0
	Female	1/1	100.0	1/1	100.0	1/1	100.0
Unknown Age	Male	0/0	0.0	0/0	0.0	0/0	0.0
	Female	2/13	15.4	9/13	69.2	10/13	76.9
Unknown Gender/Age		0/3	0.0	1/3	33.3	3/3	100.0
Total		160/651	24.6	582/651	89.4	459	71.0

Table 2: Observed Prevalence in Pregnant Women:

Prevalence of Analytes in Pregnant Women									
	Toxoplasma		Rubella		CMV		HSV-1		HSV-2	
Age	Pos/ Total	% Prevalence	Pos/ Total	% Prevalence	Pos/ Total	% Prevalence	Pos/ Total	% Prevalence	Pos/ Total	% Prevalence
16-19	2/23	9.2	23/23	100.0	16/23	69.6	15/23	65.2	3/23	13.0
20-29	12/131	27.0	35/39	89.7	104/131	79.4	92/124	74.2	51/124	41.1
30-39	10/37	55.6	127/129	28.4	26/37	70.3	30/36	83.3	14/36	38.9
40-49	5/9	8.3	9/9	100.0	5/9	55.6	8/9	88.9	7/9	77.8
Total	29/200	14.50	194/200	97.90	151/200	75.50	160/200	80.00	75/200	37.50

Table 3: Observed Prevalence in Sexually Active Adults:

		HSV-1		HSV-2	
Age	Gender	Pos/Total	% Prevalence	Pos/Total	% Prevalence
17-19	Male	0/0	0.0	0/0	0.0
	Female	27/38	71.1	6/38	15.8
20-29	Male	6/23	26.1	1/23	4.3
	Female	180/246	73.2	57/245	23.3
30-39	Male	10/16	62.5	3/15	20.0
	Female	165/187	88.2	52/187	27.8
40-49	Male	10/11	90.9	3/11	27.3
	Female	31/44	70.5	16/44	36.4
50-59	Male	5/10	50.0	5/10	50.0
	Female	8/11	72.7	6/11	54.5
60-69	Male	4/6	66.7	2/6	33.3
	Female	4/4	100.0	2/4	50.0
Total		450/596	75.5	153/594	25.8

Table 4: HSV Hypothetical Predictive Values by Prevalence:

	Sexually Active Adults				Expectant Mothers			
	HSV-1		HSV-2		HSV-1		HSV-2	
Prevalence	PPV	NPV	PPV	NPV	PPV	NPV	PPV	NPV
50.0%	94.8%	98.5%	93.7%	96.8%	87.0%	99.2%	92.9%	97.0%
40.0%	92.4%	99.0%	90.9%	97.8%	81.7%	99.5%	89.7%	98.0%
30.0%	88.7%	99.4%	86.5%	98.6%	74.2%	99.6%	84.9%	98.7%
25.0%	85.9%	99.5%	83.2%	98.9%	69.1%	99.7%	81.4%	99.0%
20.0%	82.0%	99.6%	78.8%	99.2%	62.6%	99.8%	76.6%	99.2%
15.0%	76.3%	99.7%	72.5%	99.4%	54.2%	99.9%	69.8%	99.5%
10.0%	67.0%	99.8%	62.4%	99.6%	42.7%	99.9%	59.3%	99.7%
5.0%	49.0%	99.9%	44.0%	99.8%	26.1%	100.0%	40.8%	99.8%

PERFORMANCE CHARACTERISTICS

1. Comparative Studies
   a. Performance in a Prospectively Collected Population ZEUS technicians evaluated the performance of the ToRCH panel using prospectively collected frozen remnant serum samples from 651 individuals for which ToRCH IgG panel (300), or testing for each of the individual analytes was ordered (351). Table 5 summarizes the results of this comparative study.

Table 5: Summary of Performance Characteristics in Individuals Undergoing ToRCH Antibody Assessment

			Predicate					
			Positive	Equivocal	Negative	Site Total	Sensitivity/Specificity	95% CI
ZEUS AtheNA Multi-Lyte ToRCH IgG Plus Test System	Toxoplasma	Positive	136	3	16	155	99.3% (136/137)	96.0% - 100%
		Equivocal	0	0	3	3		
		Negative	0	1	490	491	95.7% (450/514)	98.0% - 99.9%
		Invalid	0	0	2	2		
		Site Total	136	4	511	651		
	Rubella	Positive	533	4	4	541	98.5% (533/541)	97.1% - 99.4%
		Equivocal	3	1	1	5		
		Negative	2	3	60	65	*87% (60/69)	76.7% - 93.9%
		Invalid	0	0	0	0		
		Site Total	538	8	65	611		
	CMV	Positive	450	6	6	462	99.6% (450/452)	98.4% - 100%
		Equivocal	1	2	4	7		
		Negative	1	0	181	182	91.3% (181/197)	87.2% - 95.3%
		Invalid	0	0	0	0		
		Site Total	452	8	191	651		

*4/4 discrepant Rubella samples that tested positive by AtheNA and negative by ELISA had low positive values for AtheNA and high negative values for ELISA. 4/4 discrepant Rubella samples which tested positive by AtheNA and equivocal by ELISA had low positive values for AtheNA and high equivocal values for ELISA.

b. **HSV-1 and HSV-2 Performance in Sexually Active Adults**

The performance of the HSV-1 and HSV-2 was evaluated in prospectively collected samples using results from 596/651 individuals between the ages of 17 and 69. Table 6 summarizes the results of this comparative study.

Table 6: Summary of Performance Characteristics in Sexually Active Adults

			Predicate					
			Positive	Equivocal	Negative	Site Total	Sensitivity/Specificity	95% CI
ZEUS AtheNA Multi-Lyte ToRCH IgG Plus Test System	HSV-1	Positive	418	0	8	426	98.6% (418/424)	97.0% - 99.5%
		Equivocal	4	0	1	5		
		Negative	2	0	163	165	94.6% (163/172	90.3% - 97.6%
		Invalid	0	0	0	0		
		Site Total	424	0	172	596		
	HSV-2	Positive	127	0	27	154	96.9% (127/131)	92.4% - 98.8%
		Equivocal	1	0	3	4		
		Negative	3	0	433	436	93.5% (433/463)	90.9% - 95.6%
		Invalid	0	0	0	0		
		Site Total	131	0	463	594*		

*2 samples were QNS for testing HSV-2

c. **Performance in Pregnant Women Population**

ZEUS Scientific, Inc. technicians internally evaluated 200 frozen remnant serum samples collected from pregnant women between the ages of 15 and 46 for which ToRCH antibody testing was requested. Table 7 summarizes the results of this comparative study.

Table 7: Summary of Performance Characteristics in a Population of Pregnant Women

			Predicate					
			Positive	Equivocal	Negative	Site Total	PPA/NPA	95% CI
ZEUS AtheNA Multi-Lyte ToRCH IgG PlusTest System	Toxoplasma	Positive	22	1	6	29	100.0% (22/22)	87.3% - 100%
		Equivocal	0	0	1	1		
		Negative	0	0	170	170	95.3% (170/178)	91.3% - 98.0%
		Invalid	0	0	0	0		
		Site Total	22	1	177	200		
	Rubella	Positive	194	0	0	194	99.0% (194/196)	96.4% - 99.9%
		Indeterminate	1	0	0	1		
		Negative	0	1	4	5	100.0% (4/4)	47.3% - 100%
		Invalid	0	0	0	0		
		Site Total	195	1	4	200		
	CMV	Positive	151	0	0	151	98.1% (151/154)	94.4% - 99.6%
		Equivocal	0	0	0	0		
		Negative	0	3	46	49	100.0% (46/46)	93.7% - 100%
		Invalid	0	0	0	0		
		Site Total	151	3	46	200		
	HSV-1	Positive	137	0	8	145	99.3% (137/138)	96.1% - 100%
		Equivocal	0	0	0	0		
		Negative	1	0	46	47	85.2% (46/54)	72.3% - 93.4%
		Invalid	0	0	0	0		
		Site Total	138	0	54	192		
	HSV-2	Positive	68	0	7	75	97.1% (68/70)	90.1% - 99.7%
		Equivocal	0	0	2	2		
		Negative	2	0	113	115	92.6% (113/122	86.5% - 96.6%
		Invalid	0	0	0	0		
		Site Total	70	0	122	192		

d. **Agreement with CDC Panel**

The performance of the ZEUS **AtheNA Multi-Lyte** ToRCH IgG Plus Test System was assessed using masked, well-characterized serum panel from the CDC. The panels consist of:

1. 70% Toxo positive and 30% Toxo negative samples.
2. 80% Rubella positive and 20% Rubella negative samples.
3. 54% CMV positive and 46% CMV negative samples.
4. 24% HSV-1 and HSV-2 dual-positive samples, 50% HSV-1 positive and 50% HSV-1 negative samples and 48% HSV-2 positive and 52% HSV-2 negative samples.

The results are presented to convey further information on the performance of the test kit. They do not imply endorsement of the assay by the CDC. Table 8 presents the results of this testing.

Table 8: Agreement with CDC Characterized Serum Panels

			CDC Result				
			Positive	**Negative**	**Site Total**	**PPA/NPA**	**95% CI**
ZEUS AtheNA Multi-Lyte ToRCH IgG Plus Test System	**Toxoplasma**	**Positive**	70	0	70	100.0% (70/70)	95.8% - 100.0%
		Equivocal	0	0	0		
		Negative	0	30	30	100.0% (30/30)	90.5% - 100.0%
		Invalid	0	0	0		
		Site Total	70	30	100		
	Rubella	**Positive**	80	0	80	100.0% (80/80)	96.3% - 100.0%
		Equivocal	0	0	0		
		Negative	0	20	20	100.0% (20/20)	86.1% - 100.0%
		Invalid	0	0	0		
		Site Total	80	20	100		
	CMV	**Positive**	52	2	54	100.0% (52/52)	94.4% - 100.0%
		Equivocal	0	0	0		
		Negative	0	46	46	95.8% (46/48)	90.2% - 100.0%
		Invalid	0	0	0		
		Site Total	52	48	100		
	HSV-1	**Positive**	50	0	50	100.0% (50/50)	94.2% - 100.0%
		Equivocal	0	0	0		
		Negative	0	50	50	100.0% (50/50)	94.2% - 100.0%
		Invalid	0	0	0		
		Site Total	50	50	100		
	HSV-2	**Positive**	48	1	49	100.0% (48/48)	94.0% - 100.0%
		Equivocal	0	0	0		
		Negative	0	51	51	98.1% (51/52)	94.3% - 100.0%
		Invalid	0	0	0		
		Site Total	48	52	100		

e. **HSV-1 and HSV-2 Performance in a Low Prevalence Population**

ZEUS Scientific internally assessed the relative specificity of HSV-1 and HSV-2 using sera from a low prevalence population. The low prevalence population was comprised of serum samples from 18 and 19 year old subjects previously tested for infections considered non-sexual in nature.

1. **HSV-1 Reactivity**: The predicate immunoblot device was positive for seven samples and negative for 60 samples. The ZEUS **AtheNA Multi-Lyte** HSV-1 & 2 IgG test system agreed with 85.7% (6/7) of immunoblot positives and 98.3% (59/60) of immunoblot negatives.
2. **HSV-2 Reactivity**: The predicate immunoblot device was positive for 0 samples and negative for 67 samples. The ZEUS **AtheNA Multi-Lyte** HSV-1 & 2 IgG Test System agreed with 100% (0/0) of immunoblot positives and 100% (67/67) of immunoblot negatives. Table 9 summarizes the study results.

Table 9: Performance in Low Prevalence Population

			Predicate					
			Positive	**Equivocal**	**Negative**	**Site Total**	**PPA/NPA**	**95% CI**
ZEUS AtheNA Multi-Lyte ToRCH IgG Test System	**HSV-1**	**Positive**	8	0	2	10	100.0% (8/8)	68.8% - 100%
		Equivocal	0	0	0	0		
		Negative	0	0	56	56	96.6% (56/58)	88.1% - 99.6%
		Invalid	0	0	0	0		
		Site Total	8	0	58	66		
	HSV-2	**Positive**	3	0	1	4	100.0% (3/3)	36.8% - 100%
		Equivocal	0	0	0	0		
		Negative	0	0	62	62	98.4% (62/63)	91.5% - 100%
		Invalid	0	0	0	0		
		Site Total	3	0	63	66		

f. **Rubella Retrospective Negative Sample Study**

ZEUS technicians internally assessed the relative specificity of Rubella using pre-selected banked samples of sera, which previously tested negative for Rubella antibody, by the predicate device

g. **Rubella Reactivity**

The predicate ELISA device was positive for zero samples and negative for 100 samples. The Rubella analyte in the ZEUS **AtheNA Multi-Lyte®** ToRCH IgG Plus Test System agreed with 100% (0/0) of ELISA positives and 100% (100/100) of ELISA negatives.

Table 10: Rubella Retrospective Negative Sample Study

		Predicate					
		Positive	**Equivocal**	**Negative**	**Site Total**	**PPA/NPA**	**95% CI**
ZEUS AtheNA Multi-Lyte ToRCH IgG Plus Test System	**Positive**	0	0	0	0	N/A	N/A
	Indeterminate	0	0	0	0		
	Negative	0	0	100	100	100.0% (100/100)	97.1% - 100%
	Invalid	0	0	0	0		
	Site Total	0	0	100	100		

2. **Precision and Reproducibility**

ZEUS technicians evaluated the assay precision and reproducibility internally and at two external clinical sites. Technicians conducted the study as follows: A panel of six samples was identified and/or prepared (by ZEUS) for use in the study based upon their activity on the ZEUS **AtheNA Multi-Lyte** Test System. Two

samples of the panel were negative, two were high positives and the other two samples were near the assay cut off for each specific target. To assess reproducibility, technicians aliquoted each sample twice, and ran each aliquot in triplicate, each day. This resulted in six results per day. Technicians repeated this for three days at each site and the resulting data used to assess precision at each facility. Table 11 summarizes the studies.

Table 11: Summary of Reproducibility ZEUS AtheNA Multi-Lyte® ToRCH IgG Plus Test System

Panel Member	Sample N	Mean AU/mL	Within-Run SD	Within-Run %CV	Between-Day SD	Between-Day %CV	Between-Run SD	Between-Run %CV	Between-Site SD	Between-Site %CV	Total SD	Total %CV
Toxo IgG Positive 1	54	747.2	52.1	7.3	61.6	8.5	34.5	4.7	71.5	8.7	157.2	8.0
Toxo IgG Positive 2	54	770.3	50.8	6.8	63.1	8.3	39.2	4.9	78.7	8.5	170.3	8.6
Toxo IgG Positive 1 (near Cut-off)	54	158.8	158.8	9.3	18.2	11.3	12.6	8.0	18.8	12.1	29.5	11.9
Toxo IgG Positive 2 (near Cut-off)	54	140.2	140.2	8.4	16.8	11.3	13.0	8.3	20.2	12.1	33.2	12.9
Toxo IgG Negative 1	54	10.5	10.5	40.9	3.8	39.7	1.3	15.0	4.3	38.4	5.0	40.7
Toxo IgG Negative 2	54	9.1	9.1	45.4	4.1	47.4	2.4	22.6	4.7	48.0	5.6	49.8
Rubella IgG Positive 1	54	119	4.6	3.9	6.3	5.0	5.0	3.7	8.7	5.3	27.6	5.7
Rubella IgG Positive 2	54	101	5.3	5.4	5.8	5.8	3.4	3.4	9.1	6.5	15.9	7.0
Rubella IgG Positive 1 (near Cut-off)	54	22	1.2	5.4	1.4	6.5	0.9	4.4	2.3	6.2	4.8	6.4
Rubella IgG Positive 2 (near Cut-off)	54	17	0.9	5.2	1.4	9.5	1.1	5.7	2.6	10.1	3.5	10.4
Rubella IgG Negative 1	54	3	0.4	20.5	1.5	21.9	0.2	8.3	0.4	21.3	1.8	19.3
Rubella IgG Negative 2	54	4	0.4	10.3	0.6	9.8	0.3	9.4	0.8	10.9	2.2	11.0
CMV IgG Positive 1	54	996.5	88.2	8.5	99.2	9.7	58.5	5.9	108.2	10.1	167.2	10.0
CMV IgG Positive 2	54	756.9	54.5	7.0	66.7	8.7	46.8	6.3	73.3	8.8	1.6.3	8.7
CMV IgG Positive 1 (near Cut-off)	54	119.2	10.1	8.2	13.2	11.1	9.6	8.3	14.9	10.9	18.3	10.1
CMV IgG Positive 2 (near Cut-off)	54	135.3	13.6	9.9	16.4	12.1	12.6	9.4	19.5	10.7	21.2	10.8
CMV IgG Negative 1	54	17.9	5.6	29.8	6.1	32.7	3.4	19.5	6.3	28.2	7.4	27.1
CMV IgG Negative 2	54	16.4	5.5	35.5	5.9	37.1	3.6	23.4	6.3	35.4	7.3	35.4
HSV-1 IgG Positive 1	54	310.1	24.2	7.9	24.8	8.1	10	3.3	31	8.9	32.3	10.1
HSV-1 IgG Positive 2	54	392.7	31.5	8.0	32.3	8.2	15.6	3.8	48.1	8.7	50.8	8.4
HSV-1 IgG Positive 1 (near Cut-off)	54	144.6	15.4	10.7	17.2	12.0	8.9	6.0	22.3	12.4	23.0	12.4
HSV-1 IgG Positive 2 (near Cut-off)	54	191.7	17.6	9.1	19.7	10.2	9.6	5.1	24.5	9.9	27.4	10.6
HSV-1 IgG Negative 1	54	26.4	3.9	15.5	3.9	15.5	1.4	5.2	4.8	16.2	6.1	16.8
HSV-1 IgG Negative 2	54	8.2	2.3	30.7	2.5	34.1	1.3	17.0	2.7	36.9	3.5	39.4
HSV-2 IgG Positive 1	54	445.7	26.9	6.1	40.1	8.9	33.8	7.4	53.4	9.2	58.1	9.1
HSV-2 IgG Positive 2	54	355.6	27.0	7.4	30.5	8.4	18.8	5.2	51.5	8	57.2	8.6
HSV-2 IgG Positive 1 (near Cut-off)	54	152.2	14.9	9.9	16.0	10.6	7.6	5.2	25.3	10.0	31.6	11.2
HSV-2 IgG Positive 2 (near Cut-off)	54	114.1	11.4	9.8	12.5	10.9	6.3	5.7	15.4	10.8	20.4	10.8
HSV-2 IgG Negative 1	54	16.9	4.0	29.7	4.3	31.3	2.0	12.1	4.3	35.8	7.0	41.0
HSV-1 IgG Negative 2	54	21.2	5.9	27.5	6.5	30.6	3.1	15.2	6.8	26.6	8.9	27.6

3. **Rubella Traceability to WHO Standard**

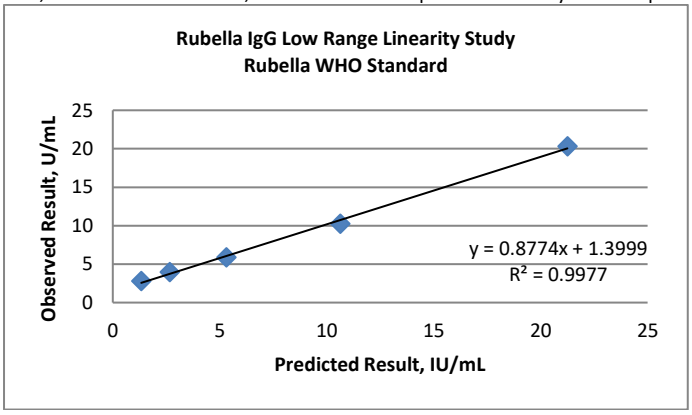
ZEUS technicians internally investigated the traceability to assess the device's correlation to the WHO Standard at the cut-off. Technicians diluted and tested the Standard in duplicate with the device on two lot numbers and the Percent Recovery calculated. At the WHO Standard dilution of 10.63, the mean of the results on Lot 1 was 9.1 with a recovery of 96%. The mean of results for Lot 2 was 10 with a recovery of 102%.

Table 12: Rubella Traceability Study

Expected Result IU/mL	**ZEUS AtheNA Multi-Lyte®** ToRCH IgG Plus Lot 1				**ZEUS AtheNA Multi-Lyte®** ToRCH IgG Plus Lot 2			
	Measured Mean		Mean % Recovery		Measured Mean		Mean % Recovery	
	Result	IU/mL	Result	IU/mL	Result	IU/mL	Result	IU/mL
1.33	3	2.7	210	205	3	2.7	231	205
2.66	4	3.6	148	137	4	3.6	146	137
5.31	6	5.5	110	103	6	5.5	105	103
10.63	10	9.1	96	86	11	10	102	94
21.25	20	18.2	96	86	20	18.2	92	86

4. **Linearity**

ZEUS technicians performed a Rubella IgG low range linearity study to demonstrate linearity across the lower range of the assay using the WHO Standard. Technicians diluted the Standard in duplicate, established the means, and calculated the percent recovery of the expected results.



5. **Rubella Performance with CDC Low Titer Sample**

Technicians assessed the Rubella performance with the CDC low titer sample (21 IU/mL). The sample was aliquoted, diluted in duplicate and tested by three technicians. Percent recovery was calculated for both neat and diluted samples.

Table 13: Rubella Performance With CDC Low Titer Sample

	CDC Low Titer Rubella Standard: 21 IU/mL					
Tech	Neat	Interpretation	% Recovery	1:2 Dilution	Interpretation	% Recovery
1	22	Positive	102%	11	Positive	102%
1	20	Positive	93%	10	Positive	99%
2	22	Positive	103%	11	Positive	109%
2	22	Positive	105%	11	Positive	104%
3	20	Positive	95%	11	Positive	101%
3	21	Positive	100%	12	Positive	111%

6. **Cross Reactivity**

ZEUS technicians performed studies to assess cross reactivity with the ZEUS **AtheNA Multi-Lyte** ToRCH IgG Plus Test System using samples sero-positive to Measles, Mumps, EBV VCA, EBNA, Rubella, VZV, ANA, CMV, Toxoplasma, HSV-1, HSV-2 and Syphilis. ELISA and micro-particle immunoassay test systems manufactured by ZEUS Scientific, Inc. for commercial distribution were used to determine the sero-positivity of the samples. This study produced no detectable cross-reactivity with samples containing these various antibodies. Refer to table 14 for a summary of the cross-reactivity study results.

Table 14: Cross Reactivity Study Results

	ZEUS AtheNA Multi-Lyte® ToRCH IgG Plus Cross Reactivity Summary (Samples Positive/Samples Tested)				
Analyte	Toxoplasma	Rubella	CMV	HSV-1	HSV-2
Measles	0/10	0/20	0/10	0/10	0/10
Mumps	0/10	0/20	0/10	0/10	0/10
Rubella	0/10	N/A	0/10	0/10	0/10
VZV	0/10	0/20	0/10	0/10	0/10
VCA IgG	0/10	0/20	0/10	0/10	0/10
EBNA IgG	0/10	0/20	0/10	0/10	0/10
HSV-1	0/10	0/20	0/10	NA	0/10
HSV-2	0/10	0/20	0/10	0/10	NA
ANA	0/10	0/4	0/10	0/10	0/10
RF	0/10	0/10	0/10	0/10	0/10
CMV	0/10	0/20	NA	0/10	0/10
Syphilis	0/10	0/10	0/10	0/10	0/10
Toxoplasma	NA	0/20	0/10	0/10	0/10

7. **Interfering Substances**

ZEUS technicians evaluated the effect of potential interfering substances on sample results generated using the ZEUS **AtheNA Multi-Lyte** ToRCH IgG Plus Test System with the following possible interfering substances based on the guidelines established in CLSI EP7-A2 (39): albumin, bilirubin, cholesterol, hemoglobin, triglycerides and intralipids. The quantity of analyte in each interfering substance is as follows:

Bilirubin: 1mg/dL (low), 15 mg/dL (high)
Albumin: 3.5 g/dL (low), 5 g/dL (high)
Cholesterol: 150 mg/dL (low), 250 mg/dL (high)
Triglycerides: 150 mg/dL (low), 500 mg/dL (high)
Hemoglobin: 20 g/dL (low), 20 g/dL (high)
Intralipid: 300 mg/dL (low), 750 mg/dL (high)

Three samples each for Toxo, Rubella, CMV, HSV-1 and 2 IgG were chosen based on their performance on the ZEUS **AtheNA Multi-Lyte®** ToRCH IgG Plus Test System: positive, borderline and negative. Technicians diluted the samples to concentrations around the cut off, and tested with the possible interfering substances at the specified high and low concentrations. All samples showed less than a 20% change in signal with the exceptions presented in table 15. Avoid the use of samples that contain elevated levels of bilirubin, albumin, cholesterol, triglycerides, hemoglobin or intralipids. The use of such samples may interfere with the outcome of the sample's result.

Table 15: Interfering Substances Study:

Analyte/Level of Sample	Potential Interfering Substance Spikes Exhibiting Change in Signal Greater than 20%											
	Bilirubin		Albumin		Cholesterol		Triglycerides		Hemoglobin		Intralipid	
	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low
Toxoplasma Positive	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	22%	<20%	<20%
Toxoplasma Borderline	<20%	<20%	-33%	-30%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%
Toxoplasma Negative	<20%	<20%	78%	89%	31%	<20%	<20%	<20%	<20%	22%	78%	<20%
Rubella Positive	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	24%	<20%	<20%
Rubella Borderline	<20%	<20%	<20%	<20%	-27%	<20%	<20%	<20%	<20%	-31%	<20%	<20%
Rubella Negative	50%	50%	<20%	-33%	<20%	-33%	-33%	-33%	<20%	-33%	<20%	<20%
CMV Positive	<20%	<20%	-27%	-25%	-32%	-31%	<20%	<20%	-33%	-35%	<20%	<20%
CMV Borderline	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	31%	24%	<20%
CMV Negative	<20%	25%	<20%	32%	<20%	<20%	<20%	<20%	<20%	25%	<20%	25%
HSV-1 Positive	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	27%	22%	<20%	<20%
HSV-1 Borderline	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%
HSV-1 Negative	<20%	<20%	41%	36%	<20%	<20%	<20%	20%	56%	26%	<20%	<20%
HSV-2 Positive	<20%	<20%	<20%	<20%	27%	<20%	24%	21%	<20%	23%	<20%	<20%
HSV-2 Borderline	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%
HSV-2 Negative	50%	41%	<20%	<20%	<20%	<20%	<20%	<20%	32%	35%	<20%	<20%

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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
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