Celiac IgA Plus Assay

A Multiplexed, Microparticle-Based Immunoassay for Antibodies towards tissue transglutaminase and Gliadin

Product Number: A61101A

INTENDED USE

The Zeus Scientific, Inc. **AtheNA Multi-Lyte®** Celiac Plus Assay is a qualitative and/or semi-quantitative assay for detection and identification of antibodies to human tissue transglutaminase (tTG) and gliadin in human serum. It is intended for use with persons of unknown risk as a test to diagnose gastrointestinal disorders, mainly celiac diseases. This test is *in vitro* diagnostic use only.

SIGNIFICANCE AND BACKGROUND

Celiac disease or gluten sensitive enteropathy is a chronic condition whose main features include inflammation and characteristic histologic "flattening" of intestinal mucosa resulting in a malabsorption syndrome (1,2). The exact etiology of the disease remains unknown but gliadin, or the alcohol soluble fraction of wheat gluten is clearly the toxic agent (3). The endomysial antigen has been identified as the protein cross-linking enzyme known as tissue transglutaminase (tTG) (4,5,6). Currently, antigen specific ELISA procedures incorporating tTG afford a reliable, objective alternative to the traditional immunofluorescent-based assays incorporating thin sections of primate esophagus as substrate (7).

Originally, a series of multiple intestinal biopsies were used to diagnose celiac and related disorders. More recently, serological testing has been suggested for screening patients with suspected gluten sensitive enteropathy as well as for monitoring dietary compliance (8, 9). Both gliadin IgA and IgG antibodies are detected in sera of patients with gluten sensitive enteropathy (10).

Gliadin IgG antibodies seem more sensitive but are less specific markers for disease compared with IgA class antibodies. Gliadin IgA antibodies are less sensitive but more specific. A sensitive screening strategy for at risk populations include testing for both gliadin IgG and IgA antibodies as a significant proportion of celiac patients are IgA deficient. Gliadin IgA antibodies are of interest for following disease activity over time and for monitoring adherence to a gluten-free diet.

PRINCIPLE OF THE AtheNA Multi-Lyte® Celiac Plus Assay

The Zeus Scientific, Inc. **AtheNA Multi-Lyte®** Plus Assay is designed to detect IgA class antibodies in human sera to tissue transglutaminase and gliadin. The test procedure involves two incubation steps:

1. Test sera (properly diluted) are incubated in a vessel containing a multiplexed mixture of the bead suspension. The multiplexed bead suspension contains a mixture of distinguishable sets of polystyrene microspheres; each set conjugated with tTG and gliadin antigens. If present in patient sera, specific antibodies will bind to the immobilized antigen on one or more of the bead sets. The microspheres are rinsed to remove non-reactive serum proteins.
2. Phycoerythrin-conjugated goat anti-human IgA is added to the vessel and the plate is incubated. The conjugate will react with human antibody immobilized on the solid phase in step 1. The bead suspension is then analysed 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).

KIT COMPONENTS

Reactive Reagents:

All reactive reagents contain sodium azide as a preservative at a concentration of 0.1% (w/v.)

1. Multiplexed bead suspension. Ready to use, 5.5 mL bottle. The suspension contains separate distinguishable 5.6 micron polystyrene beads that are conjugated with the following antigen proteins: tTG, and gliadin. The bead mix 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.
2. Phycoerythrin conjugated goat anti-human IgA (α chain specific). Ready to use, 30 mL amber bottle.
3. Human positive serum control. One, 0.2mL vial.
4. Human negative serum control. One, 0.2mL vial.
5. SAVe diluent® (sample diluent). One 50 mL bottle containing phosphate-buffered-saline. Ready to use. NOTE: The diluent will change color in the presence of serum.
6. Wash Buffer Concentrate: Dilute 1 part concentrate + 9 parts deionized distilled water. One bottle containing 10X concentrate of phosphate buffered saline.

Non-reactive Components:

1. One 96-well dilution plate
2. One 96 well filtration plate
3. Data Labels: One label is adhered to the inside lid of the kit box and a second label is inside the kit box.
4. Package Insert providing instructions for use

5. Calibration CD: a compact disc that includes all lot-specific kit calibration values required for specimen analysis and assay quality control.

Materials required but not provided:

1. AtheNA Multi-Lyte® System (Luminex® instrument)
2. Pipettes capable of accurately delivering 10 to 200 µL
3. Multichannel pipette capable of accurately delivering (10 to 200 µL)
4. Reagent reservoirs for multichannel pipettes
5. Disposable pipette tips
6. Laboratory timer to monitor incubation steps
7. Small bath sonicator
8. Plate shaker capable of shaking at 800 RPM (optional for mixing)
9. Vacuum aspirator and vacuum manifold for washing the microspheres

Procedural Precautions:

1. Dilution or adulteration of these reagents may generate erroneous results.
2. Reagents from other sources or manufacturers should not be used.
3. 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.
4. To optimize read times the bead suspension must be thoroughly mixed just prior to use. The most effective means to re-suspend the beads is to first vortex the bead suspension for approximately 30 seconds followed by sonication of the bead suspension for 30 seconds in a small bath sonicator.
5. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
6. Avoid microbial contamination of reagents. Incorrect results may occur.
7. Cross contamination of reagents and/or samples could cause erroneous results.
8. Strict 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.
9. Avoid splashing or generation of aerosols.
10. Treat the waste solution with 10% household bleach (0.5% sodium hypochlorite). Avoid exposure of reagents to bleach fumes.
11. 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 kit.

WARNINGS

1. For *in vitro* diagnostic use only.
2. **CAUTION! POTENTIAL BIOHAZARD:** The controls contain human source material. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by FDA-approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled 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 (11); and OSHA's Standard for Bloodborne Pathogens (12). The **AtheNA Multi-Lyte** Plus Assay conjugated microspheres do not contain viable organisms. However, the reagent should be considered a **POTENTIAL BIOHAZARD** and handled accordingly.
3. Caution: Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the build-up of azides if disposal into a drain is in compliance with Federal, State and local requirements.
4. Normal precautions exercised in handling laboratory reagents should be followed when performing the **AtheNA Multi-Lyte** Plus Assay. 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. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes. Do not breathe vapor. Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner, and in compliance with all Federal, State and local requirements.

STORAGE CONDITIONS

1. Store the unopened kit at 2-8°C.
2. Multiplex bead suspension: Store at 2-8°C. After re-suspending the beads, remove only the required amount of solution to analyze the specimens to be tested and return the unused portion to storage at 2-8°C.
3. Phycoerythrin conjugated goat anti-human antibody: Store at 2-8°C.
4. Human controls: Store at 2-8°C.
5. SAVe diluent:: Store at 2-8°C.
6. Wash Buffer Concentrate (10X). Store between 2-25°C. Diluted Wash Buffer (1X) is stable at room temperature (20-25°C) for up to 7 days or for 30 days at 2-8°C.

SPECIMEN COLLECTION

It is recommended that specimen collection be carried out in accordance with NCCLS/CLSI document M29: Protection of Laboratory Workers from Infectious Disease (13). No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.

Only freshly drawn and properly stored sera obtained by approved aseptic venipuncture procedures should be used in this assay. No anticoagulants or preservatives should be added. Do not use hemolyzed, icteric, lipemic, or bacterially contaminated sera. Store

sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored at 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. Specimen containers should be tightly sealed before storage. For freezing, the use of self-sealing, air tight tubes is recommended. Whenever possible, avoid the use of self-defrosting freezers because of the danger of desiccation of specimens.

ASSAY PROCEDURE

Set-up of the Assay:

Remove the individual components from storage and allow them to warm to room temperature (20-25°C). 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; the Positive Control should be tested in well B1. Each control and sample requires one microwell for processing.

Note 1. To optimise read times the bead suspension must be thoroughly mixed just prior to use. The most effective means to resuspend the beads is to first vortex the bead suspension for approximately 30 seconds and then sonicate for approximately 30 seconds in a small bath sonicator

Note 2. 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 RPM 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.

Serum Incubation

1. Prepare a 1:21 dilution of the Negative Control, the Positive Control and each of patient sera. (***Example****: Combine 10µL of serum with 200µL of SAVe Diluent*). 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. Mix according to **Note 2** above.
2. After determining the total number of wells to process, use a multichannel or a repeating pipette to dispense 50 µL of the bead suspension into each of the wells of the filtration plate (re-suspend beads according to **Note 1**).
3. 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 is thoroughly mixed. Mix according to **Note 2** above.
4. Incubate the plate at room temperature (20-25°C) for 30 +/- 10 minutes.
5. After the incubation, rinse the beads by vacuum filtration:
    a. Place the filtration plate on the vacuum manifold and remove the solution, leaving the beads behind.
    b. Turn off the vacuum and add 200 µL of Diluted Wash Buffer (1X).
    c. Apply the vacuum and remove the solution.
    d. Repeat steps 5.b and 5.c for a total of three rinses with the Diluted Wash Buffer (1X).
6. Following the final wash gently blot the bottom of the filter plate and allow plate to air dry for 3-5 minutes before proceeding to the next step.

Conjugate Incubation

1. Add 150µL of the Conjugate solution to each well at the same rate and in the same order as the specimens were added. For proper performance, it is important that the conjugate solution and bead suspension is thoroughly mixed. Mix according to **Note 2** above. As an option, while mixing the conjugate and the beads, one may transfer the bead/conjugate mixture to empty wells of a polystyrene reaction plate.
2. Incubate the plate at room temperature (20-25°C) 30 +/- 10 minutes.

Specimen Analysis

1. **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.
2. Set the **AtheNA Multi-Lyte®** instrument to analyse the reactions by selecting the Celiac IgA assay template. Refer to the operator's manual for details regarding the operation of the **AtheNA Multi-Lyte®** instrument.
3. 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.

ABBREVIATED ASSAY PROTOCOL:

Step	Procedure
1	Dilute specimens 1:21 in SAVe Diluent™. Mix well.
2	Combine 50 µL of bead suspension and 10 µL of diluted specimen in an empty well. Mix well.
3	Incubate at room temperature for 30 +/- 10 minutes
4	Rinse the microspheres 3X with Diluted Wash Buffer (1X).
5	Gently blot the bottom of the plate and air dry for 3-5 minutes.
6	Add 150 µL of conjugate to each well. Mix well and transfer to a reaction plate (optional).
7	Incubate at room temperature for 30 +/- 10 minutes.
8	Shake plate (optional).
9	Read results within 60 minutes.

CONVERSION OF FLUORESCENCE TO UNIT VALUES

A. Calculations:

1. Assay Calibration

The **AtheNA Multi-Lyte®** Celiac IgA Plus Assay 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 Scientific, Inc. These values are lot specific and are encoded within the lot specific Calibration CD included in the kit box.

2. Analyte Cut Off Values

Each analyte of the **AtheNA Multi-Lyte®** Assay has an assigned cut off value. Cut off values are determined by Zeus Scientific, Inc. for each kit lot, and are encoded within the lot specific Calibration CD included in the kit box.

3. Calculations

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.

B. Quality Control

1. Each time the assay is run it is necessary to include the Negative Control (in well A1) and the Positive Controls (in well B1). The Positive and Negative Controls are intended to monitor for substantial reagent failure.
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 Positive Control 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 multiplexed bead suspension.
    c. The Positive Control must be positive for both tTG and Gliadin In addition to the qualitative outcome; the Positive Control must meet the predetermined ranges for activity for both tTG and Gliadin. These ranges are lot specific, are encoded on the lot-specific CD and are automatically assessed by the AtheNA Multi-Lyte system®.
    d. The ranges for PC and calibration calculations 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 software and then clicking "Control Upper/Lower Limits".
    e. If any of the above criteria are not met, **do not report the patient results**. The **entire run** will be considered invalid and should be repeated.
4. 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® Plus Assay. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organisations. External controls must be representative of normal human serum since AtheNA'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. It is recommended that the user refer to NCCLS/CLSI EP12-A and 42 CFR 493.1256 for guidance on appropriate QC practices (14).

C. Interpretation of Results:

Cutoff Determination: The cut off for each assay was established using a negative population for each marker. The AtheNA results were determined for this population, and the cut off was set at approximately the mean plus three times the standard deviation. Based upon the results of this testing, the manufacturer has established the following guidelines for interpretation of patient samples.

Celiac Analyte Interpretation:

Unit Value	Result	Interpretation
< 100 AU/mL	Negative	An AtheNA result of < 100 AU/mL for tTG, and gliadin indicates no detectable IgA antibodies to that particular marker and should be reported as non-reactive for IgA. If both markers are negative and Celiac disease or gluten sensitive enteropathy is suspected, a second sample should be collected and tested no less than one to two weeks later.
100-120 AU/mL	Equivocal	Specimens with AtheNA results in the equivocal range (100 to 120 AU/mL) for tTG, and gliadin should be tested by an alternate serologic procedure. Alternatively, a second freshly collected specimen sample should be collected and tested.
>120 AU/mL	Positive	Patient sample contains antibody to tTG, and/or gliadin. A positive test result presumes Celiac disease or gluten sensitive enteropathy. Other serology assays should be performed to confirm serological status.

LIMITATIONS

1. The **AtheNA Multi-Lyte®** Test System is a diagnostic aid and by itself not a diagnostic. Test results should be interpreted in conjunction with the clinical evaluationand the test results of other diagnostic procedures.
2. Due to the homogeneous nature of this assay, hemolytic, icteric, or lipemic samples may interfere with the outcome of this assay. Additionally, specimens with abnormal IgG concentrations may interfere with the outcome of this assay. Use of these types of specimens should be avoided.
3. The results of specimens from immunesupressed patients may be difficult to interpret.
4. The test results should be evaluated with in relation to patient symptoms, clinical history, and other laboratory findings to establish a diagnosis.

Expected Results

Studies being conducted

Performance Characteristics

Studies being conducted

Cross Reactivity:

Studies being conducted

References:

1. Meuweisse G. Diagnostic criteria in celiac disease. Acta Paediatr Scand 59: 461, 1970.
2. Walker-Smith JA, Guandalin S, Schmitz J, Shmerling D H, Visakorpi J K, Revised criteria for diagnosis of celiac disease: Report of working group of European Society of Pediatric Gastroenterology and Nutrition (ESPGAN). Arch Diseases of Childhood 65: 909-911, 1990.
3. Unsworth F J. Serologic diagnosis of Gluten sensitive enteropathy. J Clin Path 49: 704-711.
4. Dieterich W, Ehnis T, Bauer M., Identification of tissue transglutaminase as the autoantigen of celiac disease. Nature Medicine #: 797-801, 1997.
5. Sollid LM, Molberg O, McAdam S, Lundin K, Autoantibodes in coeliac disease: tissue transglutiminase- guilt by association? Gut 41: 851-852 1997.
6. Sugai E, Tissue transglutinase antibodies in celiac disease: assessment of a commercial kit Am J Gastro 95: 2318-22, 2000.
7. Sblattero D, et al. Human recombinant tissue transglutaminase ELISA: an innovative diagnostic assay for celiac disease. Am J Gastroenterology 95: 1253, 2000.
8. McMillan S A, Haughton D J, Predictive value of coeliac disease of antibodies to gliadin, endomysium and jejunum in patients attempting for jejunal biopsy. BMJ 303: 1163-1165, 1991.
9. Lindh E, Ljunghall S, Screening for antibodies against gliadin in patients with osteoporosis. J Intern Med 241: 403-506, 1992.
10. Chorzelski T Pbeutner E H, Zalewski T K editors: Serologic diagnosis of celiac disease, Boca Raton (FL): CRC Press 1990.
11. U.S. Department of Health and Human Services. Public Health Service. Centers for Disease Control and Prevention and National Institutes of Health. U.S. Government Printing Office, Washington D.C., 4th Ed., 1999.
12. U.S. Department of Labor, Occupational Safety and Health Administration; Occupational Exposure to Bloodborne Pathogens, Final Rule. Fed.Register 56:64175-64182, 1991.
13. Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids and Tissues; Approved Guideline. NCCLS/CLSI Document M29, Vol.17(12), 1997.
14. CLSI. User Protocol for Evaluation of Qualitative Test Performance: Approved Guideline. CLSI document EP-12-A (ISBN 1-56238-468-6). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2002.

Marketed exclusively in the USA by:

inverness medical
professional diagnostics

2 Research Way
Princeton, NJ 08540

For Customer Service (USA):
1-800-257-9525 - enter #1
For Technical Service (USA):
1-800-257-9525 - enter #2
Fax: 1-800-532-0295

Manufactured by:

ZEUS Scientific
Diagnostic innovation. Worldwide application.

CE

Zeus Scientific, Inc.
P.O. Box 38, Raritan, New Jersey 08869, USA
For Technical Service call Toll Free: 800-286-2111
Phone: 908-526-3744 * FAX: 908-526-2058
e-mail: info@zeusscientific.com

Zeus' Authorized Representative:
Emergo Europe, Molenstraat 15, 2513 BH, The Hague
The Netherlands

AtheNA Multi-Lyte® Celiac IgA Plus/Issue Date: 2008-09-02/R2785PE/FCD-0567