510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE

A. 510(k) Number:
K033977

B. Analytes:
Autoantibodies to thyroid peroxidase (TPO) and thyroglobulin (Tg)

C. Type of Test:
Homogeneous, microparticle immunoassay (flow-cytometry)

D. Applicant:
Zeus Scientific, Inc.

E. Proprietary and Established Names:
Zeus Scientific, Inc. AtheNA Multi-Lyte™ TPO/Tg IgG Test System

F. Regulatory Information:

1. Regulation section:
   21 CFR §866.5870 Thyroid Autoantibody Immunological Test System
2. Classification:
   Class II
3. Product Code:
   JZO System, Test, Thyroid Autoantibody
4. Panel:
   Immunology 82

G. Intended Use:

1. Intended use(s):
   The Zeus Scientific, Inc. AtheNA Multi-Lyte™ TPO/Tg Test System is intended for the quantitative detection of IgG class antibody to 2 separate thyroid antigens (thyroid peroxidase (TPO) and thyroglobulin (Tg)) in human serum. The test system is intended to be used as an aid in the diagnosis of various autoimmune thyroid diseases. This test is for *In Vitro* diagnostic use only.
2. Indication(s) for use:
   The Zeus Scientific, Inc. AtheNA Multi-Lyte™ TPO/Tg Test System is intended for the qualitative and quantitative detection of IgG autoantibodies to human thyroid peroxidase and/or human thyroglobulin in human serum. The results of this serological test together with other clinical findings may aid in the diagnosis of thyroid diseases. This test is for in vitro diagnostic use
3. Special condition for use statement(s):
   The device is for prescription use only.
4. Special instrument Requirements:

AtheNA Multi-Lyte instrument

H. Device Description:

The AtheNA Multi-Lyte TPO/Tg IgG assays consist of:
-multiplexed bead suspension containing 5.6 micron polystyrene beads conjugated with thyroid peroxidase or thyroglobulin. 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;
- phycoerythrin conjugated goat anti-human IgG (γ-chain specific);
- human positive and negative serum controls; and
- sample diluent.

I. Substantial Equivalence Information:

1. Predicate device name(s):
   Zeus Scientific, Inc. TPO IgG ELISA Test System and Zeus Scientific, Inc. Tg IgG ELISA Test System
2. Predicate K number(s):
   k000362 and k000363
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Indications for Use	For the qualitative and quantitative detection of TPO and Tg IgG class antibody in human serum	Same
Conjugate	Polyclonal goat anti-human IgG (γ chain specific)	Same
Sample matrix	Serum	Same
Differences		
Item	**Device**	**Predicate**
Assay principle	Microparticle-based immunoassay (flow-cytometry)	ELISA
Solid phase	Polystyrene microspheres	Polystyrene microwells
Conjugate label	Phycoerythrin	Horse radish peroxidase
Conjugate signal	Fluorescence	Optical density
Interpretation of results	TPO and Tg: <100 IU/mL = negative; 100 to 120 IU/mL = equivocal; > 120 IU/mL = positive	TPO: <25 IU/mL = negative; 25-30 IU/mL = equivocal; >30 = positive Tg: <40 IU/mL = negative; 40-50 IU/mL = equivocal; 51-80 IU/mL = weak positive; >80 IU/mL = strong positive

J. Standard/Guidance Document Referenced (if applicable):
None referenced

K. Test Principle:
The Zeus Scientific, Inc. AtheNA Multi-Lyte™ anti-thyroid test system is designed to detect IgG class antibodies in human sera to TPO and Tg. 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 beads coated with human TPO or Tg. If present in the patient sera, specific antibodies will bind to the immobilized antigen. The microspheres are rinsed to remove non-reactive serum proteins; 2) Phycoerythrin-conjugated goat anti-human IgG (γ-chain specific) 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). The test principle and performance of the microparticle-based immunoassay (flow-cytometry) for the AtheNA Multi-Lyte™ instrument was supported in k011244 and k021103.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

To evaluate both intra-assay and inter-assay reproducibility, six specimens were tested. On each day of testing, each sample was diluted twice and then loaded for four replicates resulting in a total of eight wells of each of the six samples. This protocol was followed for three days. These results were then used to calculate mean IU/mL values, standard deviation, and percent CV. Specimens were selected in such a way that resulted in two of them being clearly negative, two being clearly positive and two were selected that were weakly positive.

Intra-assay – anti-TPO

Intra-assay %CVs for the two high positive samples ranged from 3.5 to 6.6%. For the low positive samples (near the cut-off) the %CVs ranged from 5.3 to 15.0%. The %CVs for the negative samples (close to 0 IU/mL) were 11.6 to 16.6%.

Intra-assay – anti-Tg

Intra-assay %CVs for the two high positive samples ranged from 3.3 to 7.8%. For the low positive samples (near the cut-off) the %CVs ranged from 5.4 to 15.5%. The %CVs for the negative samples (close to 0 IU/mL) were 9.3 to 16.3%.

Inter-assay – anti-TPO

Inter-assay assay %CVs for the two high positive samples were 4.3 and 5.3%. For the low positive samples (close to the cut-off) the %CVs were 10.7 and 12.7%. The two negative samples showed %CVs of 21.9 and 19.2%.

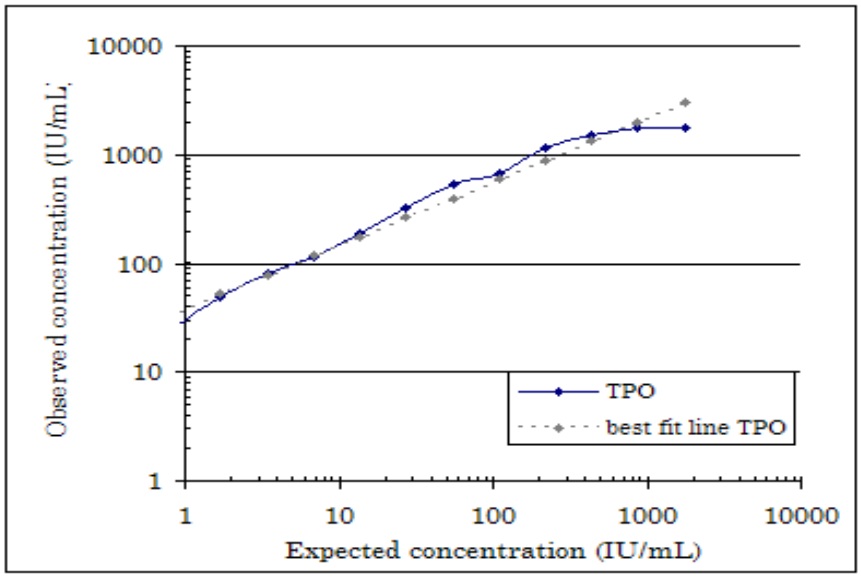
Inter-assay – anti-Tg

Inter-assay assay %CVs for the two high positive samples were 4.0 and 5.9%. For the low positive samples (close to the cut-off) the %CVs were 12.4 and 18.5%. The two negative samples showed %CVs of 13.7 and 15.7%.

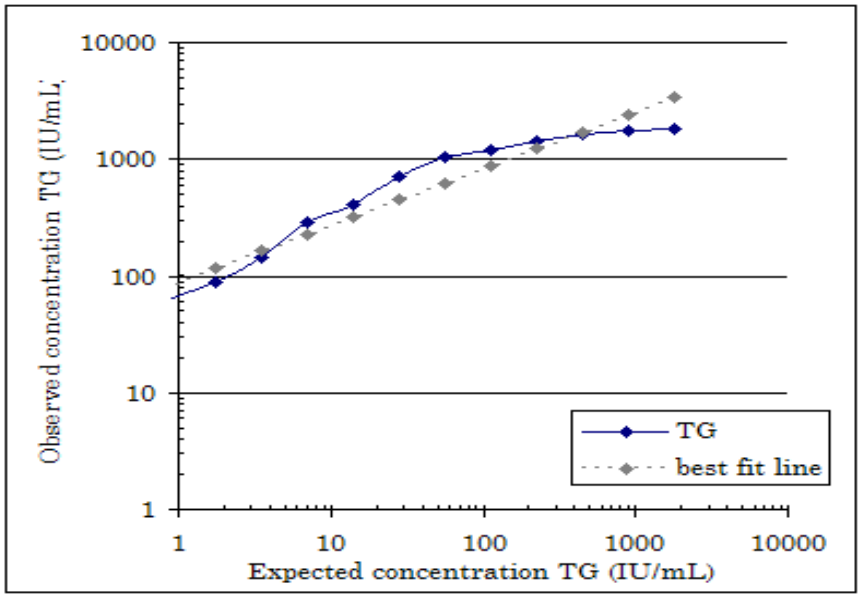
b. *Linearity/assay reportable range:*

Linearity was assessed by assaying two-fold serial dilutions of a strong positive serum. When actual concentrations were plotted against expected concentrations, non-linearity at the upper end of the assay range was observed for both assays. These type of results were as expected for assays of this type in which the amount of fluorescence changes with the patient antibody concentration but the change is not directly proportional to the quantity of autoantibody present on the bead i.e. doubling of antibody concentration does not double reactivity.

AtheNA Multi-Lyte™ anti-TPO



AtheNA Multi-Lyte™ anti-thyroglobulin



c. *Traceability (controls, calibrators, or method):*
Both assays were calibrated to International Reference Materials provided by the WHO: WHO 66/387 for anti-TPO and WHO 65/93 for anti-Tg.

d. *Detection limit:*
Not furnished but not relevant for this assay.

e. *Analytical specificity:*
The Multi-Lyte™ TPO/Tg Test System was evaluated for potential cross reactivity to other antibodies and interference from serum components. For this study, a total of 39 specimens were evaluated. Nineteen specimens were positive for various autoimmune and infectious disease antibodies. Of the 19 evaluated, 1 was reactive on the Multi-Lyte™ anti-Tg assay. The same sample was not reactive by ELISA. There were a total of 20 specimens evaluated which contained potentially interfering substances. These 20 specimens contained either abnormal levels of hemolysis (n=5), bilirubin (n=5), above normal IgG concentration (n=5), or above normal lipid levels (n=5). Two of the hemolyzed specimens were positive on the anti-TPO assay and 3 were positive on the anti-Tg assay. Two specimens with high IgG levels were positive on the anti-TPO assay (also positive by ELISA) and 1 sample was positive on the anti-Tg assay (negative by ELISA). One sample with elevated lipids was positive on the anti-TPO assay.

f. *Assay cut-off:*
The study included 150 specimens from normal blood donors. The samples were tested and the mean fluorescence and standard deviation were determined for this population. The cut-offs were arbitrarily set

equal to the mean plus three times the standard deviation. The study also included 300 specimens from patients diagnosed with an autoantibody disorder associated with anti-thyroid antibody. For the anti-TPO assay, in the normal blood donor group, 3 of the specimens were invalid on the assay, reducing the total to 147 normal specimens. Of the 147 remaining specimens, 137/147 (93.2%) were negative, 3/147 (2.0%) were equivocal and 7/147 (4.8%) were positive. The numerical results of this population ranged from 4 IU/mL to 1183 IU/mL with a mean result of 53 IU/mL and a median result of 15 IU/mL. In the clinical specimens, 0/300 (0%) were negative. Numerical results of this population ranged from 643 to 1207 IU/mL (all high value samples) with a mean of 972 IU/mL and a median result of 978 IU/mL. For the anti-Tg assay, in the normal blood donor group, 3 of the specimens were invalid on the assay, reducing the total to 147 normal specimens. Of the 147 remaining specimens, 133/147 (90.5%) were negative, 3/147 (2.0%) were equivocal and 11/147 (7.5%) were positive. The numerical results of this population ranged from 15 IU/mL to 976 IU/mL with a mean result of 78 IU/mL and a median result of 44 IU/mL. In the clinical specimens, 0/300 (0%) were negative. Numerical results of this population ranged from 428 to 1968 IU/mL (all high value samples) with a mean of 1153 IU/mL and a median result of 1188 IU/mL.

2. Comparison studies:
    a. *Method comparison with predicate device:*
    There were a total of 750 specimens tested in the comparative studies. The group included 300 specimens previously sent to a lab for routine thyroid autoantibody testing, 300 disease-state specimens from clinically diagnosed patients with thyroid autoimmune disorders and 150 normal donor sera. For anti-TPO comparison yielded an overall agreement of 95.6% (674/705) with 5 samples omitted from the calculations for invalid results on the instrument and 40 equivocal results omitted. For the anti-Tg assay the comparison yielded an overall agreement of 98.8% (710/719) with 5 samples omitted due to invalid instrument results and 26 equivocal results omitted.

    b. *Matrix comparison:*
    Serum is the only recommended matrix for both assays.

3. Clinical studies:
    a. *Clinical sensitivity:*
    Clinical sensitivity for the new assay was determined by testing 300 clinically defined serum samples from patients diagnosed with an autoimmune thyroid disorder. In this group, all 300 were positive for TPO antibody.

b. Clinical specificity:
Clinical specificity was determined using 150 normal blood donors. Three of these specimens yielded invalid results leaving 147. Of the remaining 147 specimens, 7/147 were positive, 3/147 were equivocal and 137/147 were negative. The clinical specificity of the test system was determined to be 93.2%. Expressed as a 95% confidence interval, the clinical specificity was determined to be 89.1 to 97.3.

c. Other clinical supportive data (when a and b are not applicable):

4. Clinical cut-off:
   See assay cut-off.

5. Expected values/Reference range:
   The expected value in the normal population is negative

M. Conclusion:

The Zeus Scientific, Inc. AtheNA Multi-Lyte™ TPO/Tg Test System is substantially equivalent to other devices regulated under 21 CFR §866.5870, product code JZO, Class II.