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

A. 510(k) Number:
k040466

B. Purpose for Submission:
The IgA assay is a modification of a previously cleared assay (k993612) to incorporate a human recombinant antigen in place of guinea pig transglutaminase. The IgG assay is a new device.

C. Analyte:
Anti-tissue transglutaminase IgA and IgG

D. Type of Test:
Semi-quantitative enzyme immunoassay

E. Applicant:
The Binding Site, Ltd.

F. Proprietary and Established Names:
BINDAZYME™ Human IgA Anti-Tissue Transglutaminase EIA Kit
BINDAZYME™ Human IgG Anti-Tissue Transglutaminase EIA Kit

G. Regulatory Information:
1. Regulation section:
   21 CFR 866.5660, Multiple Autoantibodies Immunological Test System
2. Classification:
   Class II
3. Product Code:
   MVM Autoantibodies, Endomysial (Tissue Transglutaminase)
4. Panel:
   Immunology 82

H. Intended Use:
1. Intended use(s):
   IgA Assay
   BINDAZYME™ Human IgA Anti-Tissue Transglutaminase EIA Kit is designed for the *in vitro* measurement of specific IgA autoantibodies against tissue transglutaminase (tTG) present in human serum, as an aid in the diagnosis of Coeliac Disease.

IgG Assay
BINDAZYME™ Human IgG Anti-Tissue Transglutaminase EIA Kit is designed for the *in vitro* measurement of specific IgG autoantibodies against tissue transglutaminase (tTG) present in human serum, as an aid in the diagnosis of Coeliac Disease.

2. Indication(s) for use:
   Same as intended use.
3. Special condition for use statement(s):
   For prescription use only
4. Special instrument Requirements:
   Plate reader capable of measuring optical densities at 450nm referenced on air.

I. Device Description:

The IgA and IgG devices are enzyme immunoassays. Components of the assays include: recombinant human transglutaminase coated microtiter plates, sample diluent, wash buffer, IgA or IgG calibrators at levels of 100, 33, 11.1, 3.7 and 1.23 U/mL, positive and negative control materials, rabbit anti-human IgA or IgG conjugate, TMB substrate, and phosphoric acid stop solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   BINDAZYME™ [IgA] Anti-Transglutaminase
2. Predicate K number(s):
   k993612
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Indications for use	Measurement of specific autoantibodies against tissue transglutaminase present in human serum as an aid in the diagnosis of celiac disease.	Same
Sample diluent, wash buffer, TMB substrate, stop solution	Same	Same
Calibrator levels (IgA)	.23, 3.7, 11.1, 33, and 100 U/mL	Same
Cut off: (IgA)	Negative - <4 U/mL Weak pos. = 4-10 U/mL Positive: >10 U/mL	Same

Differences		
Item	**Device**	**Predicate**
Calibrator levels (IgG)	1.23, 3.7, 11.1, 33, and 100 U/mL	No IgG calibrators
Antigen	Recombinant human tissue transglutaminase	Purified guinea pig tissue transglutaminase
Isotype detected	IgA or IgG separately	IgA only
Cut-off (IgG)	Negative = < 6 U/mL Weak pos. = 6-9 U/mL Positive = >9 U/mL	IgG not detected

K. Standard/Guidance Document Referenced (if applicable):
None provided.

L. Test Principle:
Microwells are pre-coated with recombinant human tTG antigen. The calibrators, controls and diluted patient samples are added to the wells. Autoantibodies specific for tTG bind to the tTG antigen in the microwells during the first incubation. After washing the wells to remove all unbound proteins, purified peroxidase labeled rabbit anti-human IgA [or IgG] is added. The conjugate binds to the captured human autoantibody and the excess unbound conjugate is removed by a further wash step. Bound conjugate is visualized with 3,3',5,5' tetramethylbenzidine (TMB) substrate which gives a blue reaction product, the intensity of which is proportional to the concentration of autoantibody in the sample. Phosphoric acid is added to each well to stop the reaction. This produces a yellow end point color which is read at 450nm.

M. Performance Characteristics (if/when applicable):
1. Analytical performance:
    a. *Precision/Reproducibility:*
       IgA device
       The inter-assay and intra assay precision compared the between and within plate reproducibility. Both sets of data were established using 3 different sera, a low normal, a medium and a moderate positive sample, all within the calibrator range of the assay. The inter-assay data compared the mean duplicate result of each of the three samples across three different kit batches. The intra-assay data shows the coefficient of variation (%CV) within a single plate based on 16 individual results for each of the three samples. Intra-batch %CV ranged from 2.7 to 10.4% and inter-batch %CV ranged from 5.5 to 7.8%.
       IgG device
       The inter-assay and intra assay precision compared the between and within plate reproducibility. Both sets of data were established using 3 different sera, a low normal, a medium and a moderate positive sample, all within the calibrator range of the assay. The inter-assay data compared the mean duplicate result of each of the three samples

across three different kit batches. The intra-assay data shows the coefficient of variation (%CV) within a single plate based on 16 individual results for each of the three samples. Intra-batch %CV ranged from 6.4 – 11.9% and inter-batch %CV ranged from 1.5 to 6.7%.

b. *Linearity/assay reportable range:*

IgA device

The measuring range of the assay is 1.23–100 U/mL. The purpose of the linearity studies was to demonstrate that all samples accurately dilute across the standard curve range and correlate to their predicted values based on dilution. Three samples were tested. Expected values ranged from <1.23 to 72.66 U/mL and percent recovery ranged from 87.3 to 124%.

IgG device

The measuring range of the assay is 1.23–100 U/mL. The purpose of the linearity studies was to demonstrate that all samples accurately dilute across the standard curve range and correlate to their predicted values based on dilution. Three samples were tested. Expected values ranged from <1.23 to 84.25 U/mL and percent recovery ranged from 82.7 to 112 %.

c. *Traceability (controls, calibrators, or method):*

There is not a recognized reference material for IgA or IgG antibodies to human tissue transglutaminase. Assay results are reported in arbitrary units.

d. *Detection limit:*

Analytical sensitivity (detection limit) was determined as the mean concentration + 2 SD given by 20 determinations of the sample diluent. For the IgA assay the studies showed the detection limit to be 0.37 U/mL and for the IgG assay it was demonstrated to be 0.24 U/mL.

e. *Analytical specificity:*

IgA device

Potentially interfering substances (bilirubin F = 18.1 mg/dL, bilirubin C = 21.6 mg/dL, hemoglobin = 480 mg/dL, and chyle 2473 units) were spiked into anti-tTG negative and positive samples and subsequently assayed to see if the interfering substance had any effect on the assay value. No significant effect on assay results was noted by addition of these substances.

IgG

The study compares a low (normal) and a high (positive) serum with and without the addition of potentially interfering serum factors. The substances are tested at elevated levels to mimic high contamination (bilirubin F= 18.6 mg/dL, bilirubin C= 22.4 mg/dL, hemoglobin= 480 mg/dL, chyle= 1670 units and rheumatoid factor= 113.5 IU/mL). The interfering substance or corresponding blank material was added at a 1:10 dilution of both the negative and the

positive sample which were then assayed to see if the interfering substance had any effect on the assay value. No significant effect on assay results was noted by addition of these potentially interfering substances.

f. *Assay cut-off:*

IgA device

Samples from 200 normal adult blood donors (100 male and 100 female subjects) were tested in singlicate for antibodies to tissue transglutaminase. On each plate of normals, 5 panel samples with known values were run to check that the calibration was accurate and thus validate the assay. Based on this study, a result of <4 U/mL is negative, 4-10 U/mL is weak positive and ≥10 U/mL is considered positive. At this cut-off, 197/200 (98.5%) of the normal sera tested were negative.

IgG device

The normal range was assessed using 200 normal blood donor samples (100 male and 100 female subjects). The samples were run in singlicate. The mean of the 200 samples was 2.05 U/mL with a standard deviation of 1.27 U/mL. The mean plus 3 SD was 5.85 U/mL with 95th percentile of 4.43 U/mL. Based on this study, a result of <6 U/mL is negative, 6-9 U/mL is weak positive and ≥ 9 is considered positive. At this cut-off, 195/200 (97.5%) of the normal sera tested were negative.

2. Comparison studies:

a. *Method comparison with predicate device:*

IgA device

Samples from known celiac and non-celiac patients (total n = 106) were assayed on the new human recombinant tissue transglutaminase antigen kit and the original guinea pig antigen tissue transglutaminase kit to show equivalence. The overall agreement was 99.1% (105/106). The one discrepant sample was weak positive by the predicate device and negative by the new device. This sample was negative by immunofluorescence assay (IFA) and biopsy.

IgG device

A mixture of celiac disease and normal samples (total n = 52) was run on the new device and the predicate device. There was an overall agreement of 86.5% (45/52). The positive agreement was 86.1% (31/36) and the negative agreement was 87.5% (14/16). One of the discrepant samples was a confirmed IgA deficient and was also endomysial antibody negative by IFA, but was biopsy positive. The low percent agreement was due to the different isotypes measured by the two assays.

b. *Matrix comparison:*

Serum is the recommended matrix for the two new assays as well as the predicate device.

3. Clinical studies:
    a. *Clinical sensitivity:*
       IgA device
       Comparison was made between biopsy results and the IgA results for 106 biopsied patients. This comparison showed an agreement of 94.3% (100/106).

       IgG device
       Comparison was made between biopsy results and the IgG assay results for 35 biopsied patients. This comparison showed an agreement of 78.8%.
    b. *Clinical specificity:*
       IgA device
       Not demonstrated
       IgG device
       Crohn's (n=50) and ulcerative colitis (n=50) samples were assayed to demonstrate clinical specificity. All 100 samples were negative at a cut-off of 6 U/mL.
    c. *Other clinical supportive data (when a and b are not applicable)*
       Not applicable.
4. Clinical cut-off:
    See assay cut-off.
5. Expected values/Reference range:
    The expected result in the normal population is negative for either IgA or IgG anti-tTG autoantibodies.

N. Conclusion:
The submitted material in this premarket notification is complete and supports a substantial equivalence decision.