

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k081674

B. Purpose for Submission:

New devices

C. Measurand:

Anti-gliadin (MGP) IgG antibody

Anti-gliadin (MGP) IgA antibody

D. Type of Test:

Semi-quantitative ELISA

E. Applicant:

The Binding Site, Ltd.

F. Proprietary and Established Names:

BINDAZYME™ Human Anti-Gliadin (MGP) IgG EIA Kit

BINDAZYME™ Human Anti-Gliadin (MGP) IgA EIA Kit

BINDAZYME™ Human Anti-Gliadin (MGP) EIA Kit (IgA or IgG)

G. Regulatory Information:

1. Regulation section:

21 § CFR § 866.5750 Radioallergosorbent (RAST) immunological test system

2. Classification:

Class II

3. Product codes:

MST, Antibodies, Gliadin

4. Panel:

Immunology 82

H. Intended Use:

1. Intended use(s):

These assays are designed for the in-vitro measurement of specific IgG or IgA antibodies against a modified gliadin peptide (MGP) in human serum, as an aid in the diagnosis of coeliac disease in conjunction with other clinical and laboratory findings.

2. Indication(s) for use:

Same as intended use.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

Microplate reader capable of measuring OD at 450 nm.

I. Device Description:

Each device contains the following: polystyrene microplate with 12 breakapart 8 well strips; Gliadin (MGP) IgG or IgA positive and negative controls, Gliadin (MGP) IgG or IgA calibrators; sample diluent; wash buffer; peroxidase-labeled IgG or IgA conjugate, TMB substrate and stop solution. Sufficient materials are

supplied to allow a maximum of 89 samples to be tested in single or 41 in duplicate, with a calibration curve and a positive and negative control yielding semi-quantitative results. If used as a qualitative assay, 93 samples in single or 45 in duplicate can be tested together with the cut-off, positive and negative control.

J. Substantial Equivalence Information:

1. Predicate device name(s):
QUANTA Lite™ Gliadin IgG II
QUANTA Lite™ Gliadin IgA II
2. Predicate K number(s):
k052142 (Gliadin IgG II)
k052143 (Gliadin IgA II)
3. Comparison with predicate:

Similarities			
Item	New Device	Predicate Device	
	BINDAZYME™ Human Anti-Gliadin (MGP) IgG or IgA EIA Kit	QUANTA Lite™ Gliadin IgA II	QUANTA Lite™ Gliadin IgG II
Method	ELISA	Same	Same
Measurement	Semi-quantitative	Same	Same
Sample	Serum	Same	Same
Positive and Negative Control	Pre-diluted human serum. Ready to use.	Same	Same
Substrate	TMB Chromogen	Same	Same
Assay Platform	96 well microtiter antigen coated plates	Same	Same
Assay washing step	Two steps	Same	Same
Detection Method	Colorimetric	Same	Same
Reading	Spectrophotometer	Same	Same

Differences			
Item	Device	Predicate	
	BINDAZYME™ Human Anti-Gliadin (MGP) IgG or IgA EIA Kit	QUANTA Lite™ Gliadin IgA II	QUANTA Lite™ Gliadin IgG II
Intended use/ Indications for Use	For the qualitative or semi-quantitative detection of IgA or IgG antibodies to modified gliadin peptide in human serum as an aid in diagnosis of celiac disease (CD)	For the semi-quantitative detection of gliadin IgA antibodies in human serum as an aid in diagnosis of CD.	For the semi-quantitative detection of gliadin IgG antibodies in human serum as an aid in diagnosis of CD.

Differences			
Item	Device	Predicate	
Antigen	Purified modified gliadin peptide	Purified gliadin peptides	Purified gliadin peptides
Sample volume required	10 µL	5 µL	5 µL
Sample diluent	Phosphate buffered saline, Tween 20	Tris buffered saline, Tween 20	Tris buffered saline, Tween 20
Wash concentrate	20X concentrate	10X concentrate	10X concentrate
Calibrators	5 calibrators: 1.23, 3.7, 11.1, 33.3, 100 U/mL	None	None
Conjugate	Horseradish Peroxidase, Goat anti-human IgG or IgA	Horseradish Peroxidase, Goat anti-human IgA	Horseradish Peroxidase, Goat anti-human IgG
Stop solution	3M Phosphoric Acid	0.344M Sulfuric Acid	0.344M Sulfuric Acid
Cut-off	10 U/mL	20 U/mL	20 U/mL
Result Interpretation	<p>Semi-quantitative test: Neg: <10 U/mL Pos: ≥10 U/mL</p> <p>Qualitative screen for IgG or IgA antibody test:</p> <p>≥ O.D. of Cut-off Control: Suspected of having anti-gliadin (MGP) antibodies. Test in the semi-quantitative assay. < O.D. of Cut-off Control: Negative</p>	<p>Neg: <20 U/mL Wk Pos: 20-30 U/mL Mod. to Strong Pos: >30 U/mL</p>	<p>Neg: <20 U/mL Wk Pos: 20-30 U/mL Mod. to Strong Pos: >30 U/mL</p>

K. Standard/Guidance Document Referenced (if applicable):

None referenced

L. Test Principle:

Microwells are pre-coated with a modified gliadin peptide. Calibrators, controls and diluted patient samples are added to the wells and antibodies recognizing the modified gliadin peptide bind during the first incubation. After washing the wells to

remove all unbound proteins, purified peroxidase labeled rabbit anti-human IgG or IgA (γ or α chain specific) conjugate is added. The conjugate binds to the captured human antibody and the excess unbound conjugate is removed by a further wash step. The 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 antibody in the sample. Phosphoric acid is added to each well to stop the reaction. This produces a yellow end point colour, which is read at 450nm.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The intra-assay precision was determined by testing six serum samples twenty times. The inter-assay precision was determined by testing six serum samples tested in duplicate six times for three/four days. Results are summarized below.

Intra-assay:

Anti-gliadin IgG		
n=20	Concentration (U/mL)	% CV
Sample 1	6.9	4.1
Sample 2	10.8	3.9
Sample 3	13.0	2.6
Sample 4	22.3	3.6
Sample 5	42.3	4.0
Sample 6	69.7	6.5

Anti-gliadin IgA		
n=20	Concentration (U/mL)	% CV
Sample 1	6.1	6.9
Sample 2	11.8	3.8
Sample 3	17.0	3.1
Sample 4	26.1	4.4
Sample 5	38.9	2.5
Sample 6	62.5	3.1

Inter-assay:

Antigliadin IgG		
n=6	Concentration (U/mL)	% CV
Sample 1	6.3	6.3
Sample 2	10.6	8.8
Sample 3	14.2	5.3
Sample 4	23.8	6.6
Sample 5	37.4	9.7
Sample 6	41.6	9.7
Antigliadin IgA		
n=6	Concentration (U/mL)	% CV
Sample 1	6.2	7.1
Sample 2	10.7	5.4
Sample 3	15.2	6.4
Sample 4	21.3	6.0
Sample 5	33.3	8.1
Sample 6	54.5	8.9

b. Linearity/assay reportable range:

Three known positive samples with different levels of Anti-Gliadin IgG or Anti-Gliadin IgA were serially diluted after the initial dilution of 1:100. The values were plotted and the correlation coefficient (r^2) were calculated. The Anti-Gliadin IgG r^2 values on the three samples were 0.9991, 0.9991, and 0.9999, and the Anti-Gliadin IgA r^2 values were 0.9990, 0.9995, and 0.9996. All samples correlate to their expected values based on the dilution and had comparable linearity.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

There is no recognized standard or reference material for Gliadin or Gliadin peptides. The calibrators, positive and negative controls are prepared in-house and arbitrary units are assigned during the development process.

Stability: The expiration date claim is 3 months for both BINDAZYME™ Human Anti-Gliadin (MGP) IgG and IgA EIA Kit. Real-time stability studies are on-going.

d. Detection limit:

The detection limit was determined by testing two low positive Gliadin IgG and IgA patient sera in 20 replicates with Gliadin IgG values of 1.2 and 1.6 and Gliadin IgA values of 1.4 and 1.8, times the lowest calibrator point value (1.23 U/mL). Statistical analysis by the Student's t test confirmed that these samples were significantly different from each other ($p < 0.0001$). The anti-Gliadin IgG or anti-Gliadin IgA antibodies were detectable at 1.23 U/mL.

e. *Analytical specificity:*

Interference by endogenous and other substances: Positive and negative serum samples were tested with the following interferents: 494 mg/dL hemoglobin, 21.6 mg/dL bilirubin C (conjugate), 19.1 mg/dL bilirubin F (Free), 1590 Units chyle, 55 IU/mL Rheumatoid Factor IgG and 50 IU/mL Rheumatoid Factor IgA. No interference by these substances was observed. The package insert states that hemolyzed, lipemic, microbially contaminated or specimens containing particulate matter should not be used in this assay.

Crossreactivity: BINDAZYME™ Human Anti-Gliadin (MGP) IgG or IgA EIA Kit was tested for crossreactivity with other antibodies with 83 clinical samples from a variety of other unrelated autoimmune diseases consisting of diabetes (21), rheumatoid arthritis (16) or with the following auto-antibodies: actin (4), AMA (6), centromere (5), LKM (4), histone (4), SSB (4), Scl-70 (4), Sm-RNP (4), GBM (4), B2GP1 IgG (2), IgA (1), IgM (1), fibrillarin (1), chromatin (1), and PCNA (1). Positive reactions were observed on 1 sample for anti-Gliadin IgG and 4 samples for anti-Gliadin IgA.

f. *Assay cut-off:*

The cut-off value of 10 U/mL for the assay was established from 200 normal adult blood donors. Gender information was available on 150 normal adult samples. The specificity for Anti-Gliadin IgG and Anti-Gliadin IgA assays were 98.5% (197/200) and 97.0% (194/200) respectively. The cutoffs were chosen to give the optimal agreement with the predicate device whilst remaining close to the mean +2SD's for normal sera.

2. Comparison studies:

a. *Method comparison with predicate device:*

Testing was performed on 183 samples (100 biopsy confirmed celiac samples and 83 unrelated autoimmune diseases). The comparative study on anti-Gliadin IgG had 100% Positive Percent Agreement (PPA) (47/47); 94.9% Negative Percent Agreement (NPA) (129/136) and 96.2% Overall Agreement (176/183) (refer to table below).

		Predicate Gliadin IgG EIA		
		Positive	Negative	Total
BINDAZYME Anti-gliadin (MGP) IgG	Positive	47	7	54
	Negative	0	129	129
	Total	47	136	183

The comparative study on anti-Gliadin IgA had 84.4% PPA (54/64); 97.5% NPA (116/119) and 92.9% Overall Agreement (170/183) (refer to table below).

		Predicate		
		Positive	Negative	Total
BINDAZYME Anti-gliadin (MGP) IgA	Positive	54	3	57
	Negative	10	116	126
	Total	64	119	183

b. Matrix comparison:

Both assays use serum as the matrix.

3. Clinical studies:

a. Clinical sensitivity and specificity:

To determine the BINDAZYME™ Human Anti-Gliadin (MGP) IgG or IgA EIA clinical sensitivity and specificity, 100 biopsy confirmed celiac samples, 83 clinical samples from other unrelated autoimmune diseases and 200 normal blood donor samples were tested. The sensitivity and specificity for Anti-Gliadin IgG were 53.0% (53/100) and 98.6% (279/283) respectively. The sensitivity and specificity for Gliadin IgA were 53.0% (53/100) and 96.5% (273/283) respectively. The new device % sensitivity was similar to the % sensitivity of the predicate ELISA devices. (refer to the two tables below).

IgG		Diagnosis		
		Positive	Negative (Disease Controls and Healthy Controls)	Total
Gliadin IgG Devices	Positive	53	4	57
	Negative	47	279	326
	Total	100	283	383

IgA		Diagnosis		
		Positive	Negative (Disease Controls and Healthy Controls)	Total
Gliadin IgA Devices	Positive	53	10	63
	Negative	47	273	320
	Total	100	283	383

b. Other clinical supportive data (when a. is not applicable):

Not applicable.

4. Clinical cut-off:

Same as assay cut-off.

5. Expected values/Reference range:

Expected values in the normal population should be negative.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.