

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY

A. 510(k) Number:

k043433

B. Purpose for Submission:

New Device

C. Measurand:

Anti- Tissue Transglutaminase

D. Type of Test:

Semi- quantitative ELISA

E. Applicant:

Hycor Biomedical Ltd.

F. Proprietary and Established Names:

AUTOSTAT™ II Anti-Tissue Transglutaminase IgG ELISA

G. Regulatory Information:

1. Regulation section:
21 CFR 866.5660, Multiple autoantibodies immunological test system
2. Classification:
II
3. Product code:
MVM, Autoantibodies, Endomysial (Tissue Transglutaminase)
4. Panel:
Immunology 82

H. Intended Use:

1. Intended use(s):
Enzyme linked immunosorbent assay method for the semi-quantitative determination of specific IgG autoantibodies to tissue transglutaminase (tTg) in human serum.
The results of the anti-tTg assay can be used as an aid in the diagnosis of Celiac Disease. Levels of these autoantibodies are one indicator in a multi-factorial diagnostic regime. In addition to the manual assay protocol, this device has been validated for use with the HYCOR HY.TEC automated EIA instrument.
For *in vitro* diagnostic use only.
2. Indication(s) for use:
Same as intended use.
3. Special conditions for use statement(s):
The device is for prescription use only.
4. Special instrument requirements:

This device has been validated for use with the HYCOR HY.TEC automated EIA instrument (k941278).

I. Device Description:

The Hycor AUTOSTAT™ II Anti-Tissue Transglutaminase IgG ELISA test is an enzyme-linked immunosorbent assay for detection of anti-tTg IgG antibodies in human serum. It consists of microplate wells coated with purified antigen, horseradish peroxidase conjugated mouse anti-human IgG, four standards, positive and negative controls, TMB substrate solution, sample diluent, wash buffer and stop solution. The conjugate, substrate and stop solution are supplied in ready to use format.

J. Substantial Equivalence Information:

1. Predicate device name(s):
AUTOSTAT™ II Anti-Tissue Transglutaminase IgA ELISA
2. Predicate 510(k) number(s):
K033744
3. Comparison with predicate:

Similarities		
Item	New Device	Predicate Device
Indications for use	The results of the anti-tTg assay can be used as an aid in the diagnosis of Celiac Disease . Levels of these autoantibodies are one indicator in a multi-factorial diagnostic regime. In addition to the manual assay protocol, this device has been validated for use with the HYCOR HY.TEC automated EIA instrument.	Same
Technology	ELISA	Same
Assay Format	Semi-quantitative	Same
Sample type and dilution	Serum at 1/100	Same
Enzyme-Conjugate	Horseradish Peroxidase	Same
Sample diluent, wash buffer, substrate and stop solution	Same	Same
Incubation times	30, 15 and 15 minutes	Same
Antigen	Recombinant tissue transglutaminase, expressed in E.coli	Same
Platform	96 well microtitre plates	Same

Differences

Item	Device	Predicate
Intended use/indications for use	IgG anti-tTg autoantibodies	IgA anti-tTg autoantibodies
Standards	10, 20, 50, 100 arbitrary u/mL	6, 12.5, 50, 150 arbitrary u/mL
Cut-off	17 u/mL	7 u/mL

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

None referenced.

L. Test Principle:

The AutostatTMII assay for detection of autoantibodies is a solid phase immunosorbent assay (ELISA) in which the analyte is indicated by a color reaction of an enzyme and substrate. The AutostatTMII wells are coated with purified antigen. Standards, controls and diluted patient sera are added to separate wells and during incubation anti-tTg IgG antibodies in the sample will bind to the antigen. After incubating at room temperature and washing away unbound material, horseradish peroxidase conjugated anti-IgG antibody is added, which binds to the immobilised antibodies. Following further incubation and washing, tetra-methyl benzidine substrate (TMB) is added to each well. The presence of the antigen-antibody-conjugate complex turns the substrate to a dark blue color. Addition of the stop solution turns the color to yellow. The color intensity is measured at 450nm by a spectrophotometer and is directly proportional to the amount of autoantibodies present in the original serum sample.

In the automated instrument, the dilutions of controls and samples are performed automatically and the assay results are calculated automatically.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

- i. Study design: Three samples (high, medium and near cut-off) were assayed in duplicates over 20 assays by the manual and by the automated method. The %CV for each duplicate in each assay was calculated, and the mean over 20 assays was taken. This is the intra-assay precision (variation). Inter-assay precision (variation) is calculated by calculating the mean concentration value between the twenty assays for each sample.
- ii. Results/Acceptance criteria: Hycor's acceptance criteria for intra-assay variation and inter-assay variation are < 10% and < 15% respectively.

Manual Method	Intra-assay Variation		
	Sample 1	Sample 2	Sample 3
CV range	0.4 – 9.6	0.4 -22.7	0.3 – 20.9
Mean CV over 20 assays	4.4	5.8	7.4
Automated Method			
CV range	0.5 – 17.2	0.4 – 12.7	0.3 – 15.3
Mean CV over 20 assays	5.4	4.1	5.0

Manual Method	Inter-assay Variation
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	Sample 1	Sample 2	Sample 3
Concentration range	15.0 - 20.3	39.3 - 52.1	62.2 – 81.2
Mean CV over 20 assays	7.4	6.5	8.2
Automated Method			
Concentration range	50.7 – 71.1	35.2 – 44.1	17.6 – 20.7
Mean CV over 20 assays	8.0	6.6	5.2

Total precision was calculated on the basis of 20 runs over 6 days. By the manual method, the total precision for sample 1 was 11.44%, for sample 2 was 14.21% and for sample 3 was 16.34%. By the automated method, the total precision for sample 1 was 13.69%, for sample 2 was 10.43% and for sample 3 was 10.83%.

b. Linearity/assay reportable range:

- i. Study design: Three samples known to contain high levels of anti-tTg IgG antibody were chosen and serially diluted to determine the linearity of the assay. From an initial dilution of 1/100, further dilutions of 1/2, 1/4, 1/6, 1/8, 1/10, 1/12 and 1/14 were made. Each dilution was run in duplicate and concentration values were plotted against dilution ratios.
 - ii. Results/Acceptance criteria: The results showed that the assay was linear up to and beyond the standard 4 at 100 u/mL. Based on this study, the dynamic range of the assay is 1 to 100 u/mL.
- c. Traceability, Stability, Expected values (controls, calibrators, or methods):*
The standards are prepared in-house. International Reference Preparation (IRP) is not available for tTg, hence, arbitrary units are assigned during the development process. These values are verified and quality control tested against previously approved batches. In addition, a panel of positive and negative samples is run to verify sensitivity of the standards.
The positive and negative controls are prepared in-house from commercially purchased sera.
The controls are quality control tested by running them at the assay dilution (1/100) on three different batches of plates using an approved batch of standards. The controls are also tested in the final assay of the complete kit prior to release to verify that the controls are within their respective acceptable ranges.
- d. Detection limit:*
The analytical sensitivity of the assay was established by calculation of the mean plus three standard deviations of a minimum of 20 replicates of the zero standard which gave a value of 0.534 u/mL for manual method and 0.598 u/ml for automated method.
- e. Analytical specificity:*
Interference by endogenous substances: No interference study data was given. The package insert states that grossly hemolysed, lipemic or microbiologically contaminated samples should not be used for this assay. Also, it states that samples with abnormally elevated levels of hemoglobin, bilirubin and especially EDTA may interfere with assay performance and accuracy.
- f. Assay cut-off:*
The cut-off value for this device was selected on the basis of statistical calculation of the mean value of 68 normal samples. The mean plus three standard deviations of this normal population was 13.5 u/ml.

The assay cut-off shown below was based on testing of 68 normal samples.

	tTG IgG (u/mL)
Negative	<17
Positive	≥17

2. Comparison studies:

a. *Method comparison with predicate device:*

- i. Study design: The manual assay was compared with predicate device which was an anti-tTg IgA kit. A total of 152 samples were assayed. 68 of the samples were from normal subjects, 47 samples were from patients with confirmed Celiac Disease and 37 samples from patients with other autoimmune conditions. Normal samples were from males and females aged between 18 and 56 years. The Celiac disease positive test panel was from a commercial supplier and included samples from males (n=13) and females (n=31) aged between 14 and 50 years.
- ii. Results: All Celiac diseased samples tested positive by the new device and by the predicate device. One normal sample and an SLE sample also tested positive by both new and predicate devices.

		Autostat II tTg IgG		
		+	-	Total
Predicate Device	+	49	0	49
	-	0	103	103

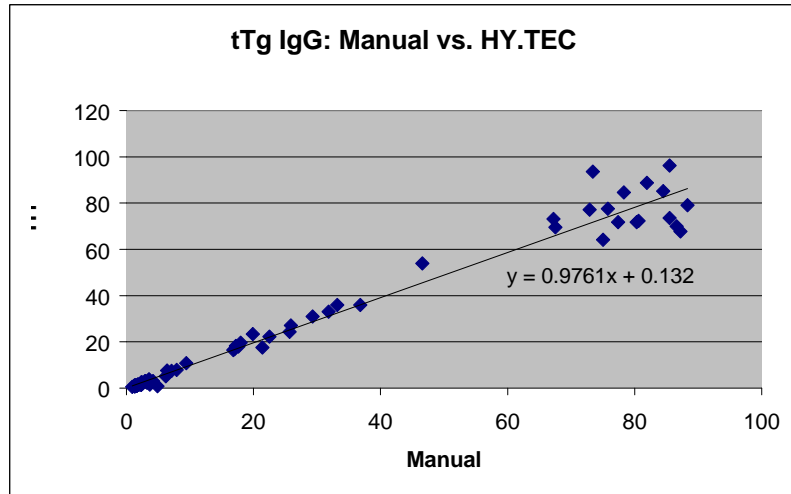
% positive agreement = 100%

% negative agreement = 100%

% total agreement = 100%

Manual and Automated correlation:

A linear regression analysis of the correlation between manual and automated tTg IgG assay was performed by running 72 samples on both systems. The correlation coefficient is 0.986. The slope is 0.9761 (95% CI: 0.95651 to 0.9957) and the intercept is 0.132 (95% CI:-0.6644 to 0.9284).



- b. *Matrix comparison:*
Not applicable.
- 3. Clinical studies:
 - a. *Clinical Sensitivity:*
A panel of 47 confirmed Celiac Disease samples was run in this study. All samples tested positive by this assay.
 - b. *Clinical specificity:*
Of a panel of 68 normal samples, 67 samples gave negative results.
 - c. *Other clinical supportive data (when a. and b. are not applicable):*
Not Applicable.
- 4. Clinical cut-off:
Same as Assay cut-off.
- 5. Expected values/Reference range:
Same as Assay cut-off.

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.