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

A. 510(k) Number:
K033744

B. Analyte:
Anti-Tissue Transglutaminase

C. Type of Test:
Semi-quantitative ELISA

D. Applicant:
Hycor Biomedical Ltd.

E. Proprietary and Established Names:
AUTOSTAT™ II Anti-Tissue Transglutaminase IgA ELISA

F. Regulatory Information:

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

G. Intended Use:

1. Indication(s) for use:
   Enzyme linked immunosorbent assay method for the semi-quantitative determination of specific IgA 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 auto-immune diseases with elevated levels of anti-tTg antibodies including Coeliac Disease. Levels of these autoantibodies are one indicator in a multi-factorial diagnostic regimen.
   This device can be used with HYCOR HY.TEC automated EIA instrument.
   For *in vitro* diagnostic use only.
2. Special condition for use statement(s):

3. Special instrument Requirements:
   This device can be used with the HYCOR HY.TEC automated EIA instrument which received FDA clearance in 1995. Dilutions of controls and patient

samples are performed automatically by the instrument. Assay results are calculated by the HY.TEC system automatically.
For manual assay, a single microplate reader with 450 nm filter or a dual microplate reader with reference filter set at 600-650 nm is used.

H. Device Description:

The device is a solid phase immunosorbent assay (ELISA) for detection of anti-tTg IgA antibodies in human serum. It consists of 96 tTg coated microwells, sheep anti-human conjugate, 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.

I. Substantial Equivalence Information:

1. Predicate device name(s):
   Eurospital Eu-Ttg IgA Umana kit
2. Predicate K number(s):
   K010625
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
	Autostat II Anti-tTg IgA	Eurospital Eu-Ttg IgA
Intended Use	Detection of auto-antibodies in human serum as an aid in the diagnosis of autoimmune disease	Same
Methodology	Solid phase immunoassay	Same
Cut-off Value	7 u/mL	Same
Differences		
Item	**Device**	**Predicate**
Application	Qualitative or Semi-quantitative	Qualitative
Assay Protocol	Manual or automated using HyCor Hy.Tec instrument	Manual assay only

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

NA

K. Test Principle:

The Autostat™ II assay for detection of autoantibodies is a solid phase immunosorbent assay (ELISA) in which the analyte is indicated by a colour reaction of an enzyme and substrate. The Autostat II wells are coated with purified antigen. On adding diluted serum to the wells, the antibodies present bind to the antigen. After incubating at room temperature and washing away unbound material, horseradish peroxidase conjugated anti-IgA antibody is added, which binds to the immobilized 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 proportional to the amount of autoantibodies present in the original serum sample.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Three samples across the assay range were tested in duplicate in a total of twenty assays and percent CV were calculated.

Manual variation

Mean conc.	Inter-assay	Intra-assay
71.5 u/mL	13.60%	5.90%
41.3 u/mL	13.50%	3.10%
7.5 u/mL	10.30%	10.30%

Automated variation

Mean conc.	Inter-assay	Intra-assay
72.8 u/mL	8.10%	5.60%
15.7 u/mL	14.50%	4.20%
7.9 u/mL	7.00%	2.80%

b. Linearity/assay reportable range:

Three samples were chosen and serially diluted to determine the linearity of the assay. The assay is linear up to 150 u/mL.

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

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 positive control is the same serum as the standard curve and the negative control is from a normal donor. 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 sensitivity of the assay was established by calculation of the mean plus three SD's of a minimum of 20 replicates of the zero standards which gave a value of 0.26 u/m for automated version and 1.18 u/mL for the manual version.

e. Analytical specificity:
Interferences and Hook Effect

Samples with abnormally elevated levels of hemoglobin, Bilirubin and EDTA may interfere with assay performance and accuracy. No hook effect was observed in this assay.

f. Assay cut-off:

u/mL	**Negative**	**Positive**
tTg IgA	< 7	> 7

2. Comparison studies:

a. Method comparison with predicate device:
The manual assay was compared with the predicate device using a panel of samples:
70 normal samples
50 confirmed celiac disease patients
17 from patients with other autoimmune conditions
24 samples from patients on which clinical information was not available (The information given relates to the designated response (DR) for either endomysial IgA or tTg IgA antibodies, as per a pre-defined reference group of laboratories).

Autostat II

		Positive	Negative
Predicate Device	Positive	68	2
	Negative	0	91

Relative sensitivity 97.1%
Relative specificity 100%
Overall agreement 98.8%

Manual and Automated Correlation

A linear regression analysis of the correlation between the manual and automated assays was performed. 55 samples were run on both systems. The correlation coefficient is 0.983.

b. Matrix comparison:
NA

3. Clinical studies:
   a. Clinical sensitivity:
   A panel of 50 confirmed Celiac disease samples gave a positive result.
   Clinical sensitivity is 100%.
   b. Clinical specificity:
   Of the 70 normal samples, 69 gave a negative result.
   Clinical specificity is 98.6%
   c. Other clinical supportive data (when a and b are not applicable):
   NA
4. Clinical cut-off:
   NA

5. Expected values/Reference range:
   The cut-off values for the device were selected on the basis of statistical calculation of the mean value of 69 of 70 normal samples. The mean plus 3 SD of the mean of this normal population was found to be 4.8 u/mL.

M. Conclusion:

Based on the review of the information provided in this 510(k), the HYCOR Autostat™ II tTg IgA appears to be **Substantially Equivalent** to devices regulated under 21CFR 866.5660, Multiple Autoantibodies Immunological Test System, Product code MVM, Class II.