

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

**A. 510(k) Number:**

k032571

**B. Analyte:**

Anti-tissue transglutaminase (tTG)

Type of Test:

Semi-quantitative- ELISA

Applicant:

IMMCO Diagnostics, Inc.

Proprietary and Established Names:

ImmuLisa Anti-Human Tissue Transglutaminase (hu-tTG) Antibody IgA ELISA

**C. Regulatory Information:**

1. Regulation section:  
866.5660 Multiple Autoantibodies Immunological Test
2. Classification:  
Class II
3. Product Code:  
MVM
4. Panel:  
Immunology (82)

**D. Intended Use:**

An enzyme linked immunosorbent assay (ELISA) for the detection and semi-quantitation of anti-human Tissue Transglutaminase IgA antibodies in human serum to aid in the diagnosis of patients with celiac disease and dermatitis herpetiformis

1. Indication(s) for use:  
An enzyme linked immunosorbent assay (ELISA) for the detection and semi-quantitation of IgA antibodies to human tissue transglutaminase, as an aid in diagnosing patients with Gluten Sensitive Enteropathy (celiac disease and dermatitis herpetiformis).
2. Special condition for use statement(s):  
Not applicable
3. Special instrument Requirements:
  - Microplate reader capable of reading absorbance values at 405 nm. If dual wavelength microplate is available, the reference filter should be set at 600-650 nm.
  - Automatic microplate washer capable of dispensing 200 uL

**E. Device Description:**

The IMMCO kit has a set of four calibrators, a positive and a negative control, microplate with individual breakaway microwells coated with hu TG antigen, anti-human Alkaline Phosphatase conjugate, serum diluent, enzyme substrate, stop solution and wash buffer. Except for the wash buffer, the reagents are ready to use

and has its own identifying color. A kit contains sufficient reagents to perform 96 determinations.

This device is a modification of a previously cleared device (K992878). The modification is a change of target antigen from a guinea pig to a human recombinant antigen. The sponsor states that guinea pig tTG antibody ELISA method has limitations with regards to sensitivity. More false negatives are observed on the guinea pig tTG antibody method. These claims of improved performance are not in the labeling or the Indications for Use. The name of the device reflects the human antigen.

**F. Substantial Equivalence Information:**

1. Predicate device name(s):
  1. IMMCO ImmuLisa Anti-tTG Antibody IgA ELISA
  2. INOVA QUANTA-Lite h-tTG (human tissue transglutaminase) IgA ELISA
2. Predicate K number(s):
  1. K992878
  2. K011566
3. Comparison with predicate:

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
	ImmuLisa hu-tTG Antibody IgA ELISA	1. ImmuLisa Anti-tTG Antibody IgA ELISA 2. INOVA QUANTA-Lite h-tTG IgA ELISA
Methodology	ELISA	Same
Detection of antibodies	tTG	Same
Quantitation	Semi-quantitative	Same
Conjugate specificity	IgA	Same
Sample	Serum	Same
<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Conjugate	Alkaline Phosphatase	1. Alkaline Phosphatase 2. Horseradish Peroxidase
Substrate	P-NPP	1. p-NPP 2. TMB
Absorbance	405 nm	1. 405 nm 2. 450 nm
Source of tTG antigen	human	1. guinea pig 2. human

**G. Standard/Guidance Document Referenced (if applicable):**

Not applicable

**H. Test Principle:**

The anti-hu tTG antibody test is performed as solid phase immunoassay (ELISA). Microwells are coated with recombinant hu tTG antigen followed by blocking the unreacted sites to reduce nonspecific binding. Controls, calibrators and patient serum samples are incubated in the antigen coated wells which allows anti-tTG antibodies present in the serum to bind. Unbound antibody and other serum proteins are removed by washing the microwells. Antibodies bound to the microwells are detected by adding enzyme labeled anti-human IgA conjugates to the wells. These enzyme conjugated antibodies bind specifically to the human immunoglobulin of the appropriate class. Unbound enzyme conjugate is removed by washing. Specific enzyme substrate (pNPP) is then added to the wells and the presence of antibodies to hu tTG is detected by a color change produced by the conversion of pNPP substrate. The reaction is stopped and the intensity of the color change, which is proportional to the concentration of the antibody, is read by a spectrophotometer at 405 nm. Results are expressed in ELISA units per milliliter (EU/mL).

## I. Performance Characteristics (if/when applicable):

### 1. Analytical performance:

#### a. Precision/Reproducibility:

The Intra-assay and Inter-assay CV of the assay were calculated based on 10 replicates.

	Inter-assay	Intra-assay
High (199 EU/mL)	6.2%	2.9%
Medium (85 EU/mL)	8.4%	6.5%
Low (14 EU/mL)	15.6%	9.6%

#### b. Linearity/assay reportable range:

To determine linearity calibrator sets were evaluated. The assay was considered linear if the calibration curves had  $r^2$  values above 0.95.

The  $r^2$  value of the curves was greater than 0.99.

The device is linear up to the value of the highest calibrator (173 EU/mL).

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

There is no reference standard or method. Positive controls and calibrators were derived from serum on patients with CD obtained from various commercial plasma centers. The sample was selected on the basis of the specific antibody reactivity and the concentration. For assignment of values, the samples were tested at various dilutions on at least two different lots of the human tissue transglutaminase antigen coated plates.

#### d. Detection limit:

Two normal patients were tested in 20 replicates. The mean + 2 SD's was calculated to determine the limit of detection (14.82 EU/mL)

#### e. Analytical specificity:

Interference - The study demonstrates that lipemic and hemolyzed sera have no significant effect in determining the levels of anti-tTG antibodies. A total of 65 disease controls from autoimmune vesicobullous such as pemphigus were tested. None were found positive.

#### f. Assay cut-off:

<20 EU/mL	Negative
20-25 EU/mL	Indeterminate (borderline)
>25 EU/mL	Positive

2. Comparison studies:

a. *Method comparison with predicate device:*

- i) ImmuLisa anti-tissue transglutaminase IgA ELISA vs. ImmuLisa human tTG IgA Antibody ELISA  
200 samples {50 normal sera, 65 disease controls (pemphigus/pemphigoid), 17 celiac disease on gluten free diet and 68 gluten sensitive enteropathy (celiac disease and dermatitis herpetiformis)}, were tested. The results are summarized below:

**ImmuLisa anti-human tTG IgA**

**ImmuLisa  
Anti-tTG  
IgA  
ELISA**

	Positive	Negative	Total
Positive	54	1	55
Negative	3	142	145
Total	57	143	200

Relative Sensitivity: 98.2%

Relative Specificity: 97.9%

Relative Agreement: 98%

- ii) INOVA anti h-tTG IgA vs. ImmuLisa human tTG IgA Antibody ELISA  
74 samples {17 disease controls (pemphigus/pemphigoid) and 57 gluten sensitive enteropathy (celiac disease and dermatitis herpetiformis)}, were tested. The results are as follows:

**ImmuLisa anti-human tTG IgA**

**INOVA  
Anti- h-tTG  
IgA ELISA**

	Positive	Negative	Total
Positive	57	1	58
Negative	1	15	16
Total	58	16	74

Relative Sensitivity: 98%

Relative Specificity: 94%

Relative Agreement: 97%

One of the negatives on the ImmuLisa human tTG kit was classified as a weak positive on the INOVA tTG test kit. The nature of the discrepant result cannot be determined as these samples were received by a reference laboratory for CD serology testing.

b. *Matrix comparison:*

Not applicable.

3. Clinical studies:

*a. Clinical sensitivity:*

Not applicable

*b. Clinical specificity:*

Not applicable

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

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

The normal range was established by testing 64 serum samples from healthy donors obtained from the local Red Cross. The mean plus 3 SD of the mean of this normal population was used to determine the cut-off between normal and abnormal specimens, and was found to be 20 EU/mL

**J. Conclusion:**

Based on the review of the information provided in this 510(k), the ImmuLisa Anti-Human Tissue Transglutaminase (hu-tTG) Antibody IgA ELISA appears to be **Substantially Equivalent** to devices regulated under 21CFR 866.5660, Multiple Autoantibodies Immunological Test System, Product code MVM, Class II.