

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY

A. 510(k) Number:

k040095

B. Analyte:

Anti-tissue transglutaminase

C. Type of Test:

Semi-quantitative, ELISA

D. Applicant:

IMMCO Diagnostics, Inc.

E. Proprietary and Established Names:

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

ImmuLisa™ Anti-Human Tissue Transglutaminase (hu-tTG) Antibody Total
IgA/IgG ELISA

F. Regulatory Information:

1. Regulation section:
21 CFR §866.5660 Multiple Autoantibodies Immunological Test
2. Classification:
Class II
3. Product Code:
MVM, autoantibodies, endomysial (tissue transglutaminase)
4. Panel:
IM (82)

G. Intended Use:

1. Intended use(s):
ImmuLisa™ Anti-Human Tissue Transglutaminase (hu-tTG) Antibody IgG ELISA is an enzyme linked immunosorbent assay (ELISA) for the detection and semi-quantitation of anti-human Tissue Transglutaminase IgG antibodies in human serum to aid in the diagnosis of celiac disease (CD) in patients with IgA deficiency.
ImmuLisa™ Anti-Human Tissue Transglutaminase (hu-tTG) Antibody Total IgA/IgG ELISA is an enzyme linked immunosorbent assay (ELISA) for the detection and semi-quantitation of anti-human Tissue Transglutaminase antibodies in human serum to aid in the diagnosis of celiac disease (CD) in patients and dermatitis herpetiformis.
2. Indication(s) for use:
Same as Intended use
3. Special condition for use statement(s):
For Prescription Use Only
4. 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.

H. Device Description:

The IMMCO kits have a set of four calibrators, a positive and a negative control, microplate with individual breakaway microwells coated with hu-tTG 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.

I. Substantial Equivalence Information:

1. Predicate device name(s):
INOVA Quanta Lite™ h-tTG IgG ELISA
IMMCO Immuglo™ Anti-Endomysial Antibody (EMA) Test System
2. Predicate K number(s):
k011570
k912551
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	ImmuLisa hu-tTG Antibody IgG ELISA	INOVA QUANTA-Lite h-tTG IgG ELISA
Intended Use	Detection and semi-quantitation of anti-human Tissue Transglutaminase IgG antibodies in human serum to aid in the diagnosis of celiac disease (CD) in patients with IgA deficiency.	Same
Methodology	ELISA	Same
Assay format	Semi-quantitative	Same
Conjugate specificity	IgG	Same
Sample	Serum	Same
Differences		
Item	Device	Predicate
Conjugate	Alkaline Phosphatase	Horseradish Peroxidase
Source of human tTG antigen	Recombinant	Native
Substrate	P-NPP	TMB Chromogen
Absorbance	405 nm	450 nm
Screening dilution	1:51	1:101

Similarities		
Item	Device	Predicate
	ImmuLisa hu-tTG Total IgA/IgG Antibody ELISA	IMMCO EMA IFA
Intended Use	For the detection and semi-quantitation of anti-human Tissue Transglutaminase antibodies in human serum to aid in the diagnosis of celiac disease (CD) in patients and dermatitis herpetiformis.	Same
Assay Format	Semi-quantitative	Same
Sample	Serum	Same
Differences		
Item	Device	Predicate
Conjugate	Enzyme labeled	Fluorescein labeled
Conjugate specificity	IgA/IgG	Polyvalent
Methodology	ELISA	Indirect immunofluorescence
Antigen	Immobilized on the microwells	Bound on tissue sections adhered to a glass slide

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

None referenced

K. Test Principle:

The anti-hu tTG antibody test is a 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-hu tTG antibodies present in the sample 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 Ig 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).

L. Performance Characteristics (if/when applicable):1. Analytical performance:a. *Precision/Reproducibility:*

Based on 10 replicates, the intra-assay and inter-assay coefficient of variation (CV) of the devices were calculated.

IgG anti-hu tTG ELISA

Specimen	Inter-assay CV	Specimen	Intra-assay CV
High (126.9 EU/mL)	8%	High (145.6 EU/mL)	7%
Med (102.3 EU/mL)	12%	Med (117.3 EU/mL)	9%
Low (48.9 EU/mL)	10%	Low (59.3 EU/mL)	8%
Cut-off (20 EU/mL)	9%	Cut-off (20 EU/mL)	10%

Anti-hu tTG Total IgG/IgG ELISA

Specimen	Inter-assay CV	Specimen	Intra-assay CV
High (126.9 EU/mL)	3.8%	High (111.1 EU/mL)	5.5%
Med (66.6 EU/mL)	5.8%	Med (97.5 EU/mL)	9.7%
Low (39.6 EU/mL)	6.4%	Low (35.5 EU/mL)	8.5%
Cut-off (20 EU/mL)	8.7%	Cut-off (20 EU/mL)	10.5%

b. Linearity/assay reportable range:

Five plates were assayed with calibrators of known values. The r-squared values of the standard curve were determined. An R^2 value greater than 0.95 is deemed acceptable. For the IgG assay, the average value was 0.9816 with no value lower than 0.9595. For the IgA/IgG assay, the average value was 0.9975 with no value lower than 0.9929. The device is linear up to the highest calibrator.

Anti-hu-tTG Antibody IgG ELISA

$$y = 0.0253x + 0.088$$

$$R^2 = 0.999$$

Anti-hu-tTG Antibody Total IgA/IgG ELISA

$$y = 0.0234x + 0.0015$$

$$R^2 = 1.000$$

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

Calibrators and controls are not traceable to any recognized standards. Calibrators and positive controls were derived from serum of patients with Celiac Disease. The negative control is serum obtained from commercial sera sources. The sera obtained are tested for clarity and reactivity on the various analyte in which sera will be used as a control.

d. Detection limit:

Not Applicable.

e. *Analytical specificity:*

Interference:

Eleven hemolytic, 11 lipemic and one icteric samples were tested. Levels of anti-hu tTG antibodies were determined. Results demonstrate that lipemic, hemolytic and icteric sera have no significant effect in determining the levels of anti-hu tTG antibodies

Cross Reactivity Studies:

A total of 30 samples, 10 each positive for antinuclear, anti-basement zone (BMZ) and anti-intercellular (IC) antibodies were tested for anti-hu tTG antibodies. None were found positive.

A total of 56 samples (patients with connective tissue disorders such as psoriasis, autoimmune bullous disorders such as pemphigous and pemphigoid) were tested for anti-hu tTG total IgA/IgG antibodies. Two were found weakly positive.

f. *Assay cut-off:*

Cut off value was established by testing 66 serum samples from apparently healthy donors obtained from the local Red Cross. The mean plus 3 SD of this normal population was found to be 20 EU/mL and is used as the cut-off.

Anti-hu-tTG Antibody IgG ELISA

Mean 0.2151 (OD)
SD 0.0928 (OD)
Cut-off 20 EU/mL (OD 0.4937)

Anti-hu-tTG Antibody Total IgA/IgG ELISA

Mean 0.2100 (OD)
SD 0.0980 (OD)
Cut-off 20 EU/mL (OD 0.505)

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

2. Comparison studies:

a. *Method comparison with predicate device:*

A total of 68 samples were tested on the Immulisa anti-hu tTG IgG antibody and its predicate device INOVA anti-h-tTG IgG ELISA. Seven of the eight samples positive on IMMCO tTG and negative on the INOVA were from patients with IgA-deficient CD with one patient having DH. The results are as follows:

INOVA

IMMCO		Pos	Neg	Total
	Pos	13	8	21
	Neg	0	47	47
	Total	13	55	68

Positive Agreement 100%
 Negative Agreement 85%
 Total Agreement 88%

A total of 200 samples were tested on the IMMCO ImmuLisa anti-hu tTG Total IgA/IgG antibody ELISA and its predicate device IMMCO EMA assay. Two of the four samples positive on the ELISA and negative on the EMA were sera from patients with DH and were weak positives. The other 2 false positives were within the Pemphigus/Pemphigoid disease population group. Three of the five samples negative on IMMCO ELISA and positive on the EMA had titers of 10 or less. The other samples in the CD subset had titers of 40 and 20 with 14.6 and 19.8 EU/mL results respectively. The results are summarized below:

IMMCO EMA

IMMCO ELISA		Pos	Neg	Total
	Pos	102	4	106
	Neg	5	89	94
	Total	107	93	200

Positive Agreement 95%
 Negative Agreement 96%
 Total Agreement 96%

- b. *Matrix comparison:*
 Not applicable

3. Clinical studies:

- a. *Clinical sensitivity:*

Anti-hu-tTG) Antibody IgG ELISA

Serum from patients with CD with and without IgA deficiency, and dermatitis herpetiformis were tested for IgG anti-hu tTG antibodies. Results are as follows:

Diagnosis	No. tested	No. positive
IgA-deficient (celiac)	15	15
IgA-deficient(non-celiac)	12	0
Dermatitis herpetiformis	22	4

Anti-hu-tTG Antibody Total IgA/IgG ELISA

Serum from patients with CD with and without IgA deficiency, and dermatitis herpetiformis were tested for IgG anti-hu tTG antibodies. Results are as follows:

Diagnosis	No. tested	No. positive
IgA-deficient (celiac)	15	15
IgA-deficient(non-celiac)	13	0
Celiac disease	94	75
Dermatitis herpetiformis	22	14

The 19 celiac samples that were negative for total anti-hu tTG antibodies also tested negative on both anti-hu tTG IgG ELISA and anti-hu tTG IgA ELISA. It is probable that these patients were on a gluten free diet.

b. Clinical specificity:

Anti-hu-tTG Antibody IgG ELISA

44 disease controls which include sera from adult patients with clinical diagnosis of blistering diseases as pemphigus, pemphigoid, and another dermatological disorder, psoriasis were tested. None were positive for IgG anti-hu tTG antibodies. In addition, of the 66 normal controls, two were weak positives.

Anti-hu-tTG Antibody Total IgA/IgG ELISA

56 disease controls which include sera from adult patients with clinical diagnosis of blistering diseases as pemphigus, pemphigoid, and another dermatological disorder, psoriasis were tested. 2 were positive for total IgA/IgG anti-hu tTG antibodies. In addition, of the 65 normal controls, 4 were weak positive.

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:

IgG anti-tTG antibodies are of interest only in Celiac patients who are IgA deficient. The incidence of IgG anti-tTG antibodies in non-immunodeficient patients is approximately 30%. The level of the IgG anti-tTG antibodies is typically lower than that of IgA; however, in IgA deficient CD patients, IgG class anti-tTG antibodies are the only type of antibodies present. The incidence of IgA deficiency in the general population is approximately 1:500. The incidence of celiac disease in the general population is approximately

1:150. The incidence of CD in IgA deficient patients is about 10 times that of the normal population.

M. Conclusion:

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