

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

A. 510(k) Number:

k102964

B. Purpose for Submission:

New Device

C. Measurand:

Anti-Tissue Transglutaminase IgA

Anti-Tissue Transglutaminase IgG

D. Type of Test:

Semi-quantitative enzyme immunoassay

E. Applicant:

Grifols USA, LLC

F. Proprietary and Established Names:

Eu-tTG® IgA and Eu-tTG® IgG

G. Regulatory Information:

1. Regulation section:

21 CFR §866.5660, Multiple autoantibodies immunological test system

2. Classification:

Class II

3. Product code:

MVM, Autoantibodies, Endomysial (Tissue Transglutaminase)

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

Same as Indications for use

2. Indication(s) for use:

The Eu-tTG IgA is an *in vitro* diagnostic enzyme immunoassay for the semi-quantitative detection of IgA specific antibodies directed against tissue transglutaminase (tTG) in human serum. It is an aid in the diagnosis of celiac disease and should be used in conjunction with other serological tests and clinical findings.

The Eu-tTG IgG is an *in vitro* diagnostic enzyme immunoassay for the semi-quantitative detection of IgG specific antibodies directed against tissue transglutaminase (tTG) in human serum. It is an aid in the diagnosis of celiac disease and should be used in conjunction with other serological tests and clinical findings.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

Microtiter plate reader capable of measuring OD at 450 and 620 nm.

I. Device Description:

Each test kit for Eu-tTG IgA & Eu-tTG IgG consists of one microtiter plate (12 strips

with 8 wells coated with the human recombinant tTG antigen), assay controls (positive and negative), a ready-to-use set of five calibrators (0, 10, 20, 50, 100 AU/mL), Horseradish Peroxidase (HRP) goat anti-human IgA or IgG conjugate, serum diluent, Tetramethylbenzidine (TMB) enzyme substrate, stop solution (0.5M H2SO4), and washing solution required for the assay.

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) number(s):
 Eu-tTG® IgA umana (k010625)
 AESKULISA® tTg G (k042644)
2. Comparison with predicate:

Similarities		
Item	Device	Predicate
	Eu-tTG® IgA (k102964)	Eu-tTG®IgA umana (k010625)
Intended Use	The Eu-tTG IgA is an <i>in vitro</i> diagnostic enzyme immunoassay for the semi-quantitative detection of IgA specific antibodies directed against tissue transglutaminase (tTG) in human serum. It is an aid in the diagnosis of celiac disease and should be used in conjunction with other serological tests and clinical findings.	Eu-tTG IgA Umana Assay is a qualitative ELISA test for the <i>in vitro</i> diagnostic detection of IgA antibody against the recombinant enzyme tissue transglutaminase (tTG) in human serum. This test is designed for use as an aid in the diagnosis of Celiac Disease.
Methodology	ELISA	Same
Analyte	Anti-human tissue transglutaminase (tTG) IgA antibodies	Same
Capture Antigen	Human recombinant tTG	Same
Detection Antibody	Goat anti-human IgA conjugate	Same
Enzyme Conjugate	Horseradish peroxidase (HRP)	Same
Substrate/Chromogen	TMB	Same
OD Reading	450 nm on spectrophotometer	Same
Positive Control	Human serum positive for tTG IgA Antibodies	Same
Negative Control	Human serum	Same
Controls	One positive control One negative control	Same
Storage	2-8°C	Same
Sample Volume Required	100 µL	Same

Similarities		
Item	Device	Predicate
	Eu-tTG® IgA (k102964)	Eu-tTG®IgA umana (k010625)
Sample Diluent	Ready-to-use	Same
Wash Solution/Buffer	20 X concentrated	Same

Differences		
Item	Device	Predicate
	Eu-tTG® IgA (k102964)	Eu-tTG®IgA umana (k010625)
Cutoff	9 AU/mL	7 AU/mL (Ages 2 to Adult); 5 AU/mL (Children < 2 years)
Linearity range	4.2-99.9 AU/mL	not applicable
Screening Dilution	1:101	1:26
Calibrators	5 Calibrators: 0, 10, 20, 50, 100 AU/mL	One calibrator: 16 AU/mL
Incubation Times	45-30-15 minutes	60-30-30 minutes
Linear Range	4.2-99.9 AU/mL	not applicable
Limit of Detection	1.6 AU/mL	not applicable

Similarities		
Item	Device	Predicate
	Eu-tTG® IgG (k102964)	AESKULISA® tTg G (k042644)
Intended Use	The Eu-tTG IgG is an <i>in vitro</i> diagnostic enzyme immunoassay for the semi-quantitative detection of IgG specific antibodies directed against tissue transglutaminase (tTG) in human serum. It is an aid in the diagnosis of celiac disease and should be used in conjunction with other serological tests and clinical findings.	The AESKULISA tTg G is a solid phase enzyme immunoassay for the semi-quantitative and qualitative detection of IgG antibodies against tissue transglutaminase (tTG) in human serum. The assay is an aid in the diagnosis of celiac disease (gluten-sensitive enteropathy) and should be used in conjunction with other serological tests and clinical findings. For <i>in vitro</i> diagnostic use only.
Methodology	ELISA	Same

Similarities		
Item	Device	Predicate
	Eu-tTG® IgG (k102964)	AESKULISA® tTg G (k042644)
Analyte	Anti-human tissue transglutaminase (tTG) IgG antibodies	Same
Capture Antigen	Human recombinant tTG	Same
Detection Antibody	Goat anti-human IgG conjugate	Same
Enzyme Conjugate	Horseradish peroxidase (HRP)	Same
Substrate/Chromogen	TMB	Same
Screening Dilution	1:101	Same
OD Reading	450 nm on spectrophotometer	Same
Positive Control	Human serum positive for tTG IgG Antibodies	Same
Negative Control	Human serum	Same
Storage	2-8°C	Same
Sample Volume Required	100 µL	Same

Differences		
Item	Device	Predicate
	Eu-tTG® IgG (k102964)	AESKULISA® tTg G (k042644)
Cutoff	20 AU/mL	15 U/mL
Calibrators	5 Calibrators: 2, 10, 20, 50, 100 AU/mL	6 Calibrators: 0, 3, 10, 30, 100, 300 U/mL
Controls: Positive Negative	40-80 AU/mL 0-5 AU/mL	One positive control One negative control One cut-off control
Linear Range	4.1-99.8 AU/mL	1-100 U/mL
Limit of Detection	1.5 AU/mL	1.0 U/mL
Sample Diluent	Ready-to-use	5x concentrated

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

CLSI EP5-A2, “Evaluation of Precision Performance of Quantitative Measurement Methods”

CLSI EP6-A, “Evaluation of the Linearity of Quantitative Measurement Procedures; A Statistical Approach”

CLSI EP7-A2, “Interference Testing in Clinical Chemistry”

CLSI EP17-A, “Protocols for Determination of Limits of Detection and Limits of Quantitation”

CLSI C28-A2, “How to Define and Determine Reference Intervals in the Clinical Laboratory”

CLSI EP9-A2, “Method Comparison and Bias Estimation Using Patient Samples”

L. Test Principle:

The Eu-tTG IgA and Eu-tTG IgG tests are sandwich type enzyme immunoassays. The wells of a microtiter plate are coated with human recombinant tissue transglutaminase. Antigen-specific antibodies in the patient serum bind to the antigens. Non-specific antibodies are removed by washing. Horseradish peroxidase labeled goat anti-human IgA or IgG are added and bind to human antibodies in the well. The excess conjugate is washed away; then a chromogenic substrate is added. After an appropriate incubation period, the OD value is measured using an ELISA microtiter plate reader capable of measuring OD at 450 nm.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The repeatability (intra-assay) precision studies were performed by testing ten serum samples, in 10 replicates in one run. Results are summarized in the following two tables:

Repeatability (Intra-assay) Precision of Eu-tTG IgA:

Sample No.	1	2	3	4	5	6	7	8	9	10
Mean (AU/mL)	86.4	59.4	38.5	37.6	28.0	13.2	6.6	4.2	2.9	2.5
SD	3.83	3.99	1.26	0.77	1.22	1.14	0.26	0.16	0.08	0.10
CV% (<10%)	4.4	6.7	3.3	2.1	4.4	8.6	3.9	3.7	2.6	4.0

Repeatability (Intra-assay) Precision of Eu-tTG IgG

Sample No.	1	2	3	4	5	6	7	8	9	10
Mean (AU/mL)	87.0	63.9	58.5	53.8	42.9	39.9	39.8	33.1	9.9	8.9
SD	3.32	2.96	5.07	4.37	3.14	2.28	3.61	2.95	0.27	0.56
CV% (<10%)	3.8	4.6	8.7	8.1	7.3	5.7	9.1	8.9	2.7	6.3

The between-days (inter-run) precision studies were performed by testing ten serum samples, in five replicates for six days using one lot of the assay kit (total of 240 samples). The results are summarized in the following two tables:

Inter-run Precision of Eu-tTG IgA

Sample No.	1	2	3	4	5	6	7	8	9	10
Mean (AU/mL)	85.1	59.9	38.2	32.6	28.2	11.4	7.1	4.0	2.7	2.7
SD	2.69	2.29	0.91	3.0	2.04	0.64	0.73	0.41	0.26	0.22
CV% (<15%)	3.2	3.8	2.4	9.2	7.2	5.6	10.2	10.2	9.7	8.0

Inter-run Precision of Eu-tTG IgG

Sample No.	1	2	3	4	5	6	7	8	9	10
Mean (AU/mL)	89.5	68.4	52.5	45.2	39.6	37.1	36.2	36.2	9.6	8.3
SD	3.03	4.74	1.82	3.24	1.46	3.07	1.85	2.24	0.44	0.66
CV% (<15%)	3.4	6.9	3.5	7.2	3.7	8.3	5.1	6.2	4.6	7.9

Inter-lot precision study was also performed by testing seven serum samples in duplicate, using 3 lots of the assay kit. The lot to lot CV was less than 11%.

Inter-lot Precision results are summarized in the table below:

Sample	1	2	3	4	5	6	7
Eu—tTG IgA							
Mean (AU/mL)	92.2	68.5	62.0	26.1	15.1	4.5	2.5
SD	5.6	2.0	6.6	1.3	0.2	0.3	0.1
%CV	6.1	2.9	10.6	5.0	1.0	5.6	5.4
Eu—tTG IgG							
Mean (AU/mL)	89.4	67.0	42.3	19.3	17.6	14.6	10.3
SD	3.75	4.7	0.9	0.8	0.3	1.6	0.7
%CV	4.2	7.1	2.1	4.0	1.5	10.6	6.6

b. Linearity/assay reportable range:

The linearity studies were assessed according to EP6-A. Three positive samples (2 samples ~ 100 AU/mL and 1 sample ~ 50 AU/mL) each for the Eu-tTG IgA and Eu-tTG IgG were used in the study. Each sample was diluted with a low concentration serum sample (around limit of detection) and tested in duplicate. The observed values were graphed against the calculated values and linear regression was performed. The results are summarized in the table below:

Test Range (AU/mL)	Slope (95% CI)	Y-intercept (95% CI)	R ²	% Recovery (observed/expected)
Eu-tTG IgA				
14.4 to 99.8	1.028 (0.918-1.139)	-4.231 (-11.15-2.69)	0.9830	86 to 103
4.4 to 92.8	0.968 (0.899-1.037)	-1.616 (-5.763-2.531)	0.9925	86 to 110
4.1 to 57.6	0.981 (0.909-1.054)	-1.154 (-3.74-1.433)	0.9906	87 to 103
Eu-tTG IgG				
4.7 to 99.9	0.933 (0.847-1.019)	-1.792 (-7.009-3.425)	0.9854	86 to 102
4.2 to 94.8	0.917 (0.860-0.974)	-1.407 (-4.831-2.016)	0.9933	85 to 95
4.5 to 56.4	1.067 (0.938-1.195)	-2.894 (-7.342-1.554)	0.9787	87 to 109

The claimed Eu-tTG IgA linear range is 4.1 to 99.8 AU/mL
 The claimed Eu-tTG IgG linear range is 4.2 to 99.9 AU/mL.

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Traceability: There is no recognized standards for this analyte.

Calibrators: Calibrators are prepared in-house from dilutions of the pooled serum of tTG antibody from patients with Celiac disease, obtained from various commercial plasma centers. Arbitrary units are assigned during the development process. As new lots of calibrators are developed, studies are performed to calibrate values against original calibrators, that are previously approved lot of calibrators. Each lot of calibrator is also tested in comparison with normal human sera, clinical samples and internal standards. The concentration values of the calibrators are as follows:

Calibrator	IgA (AU/mL)	IgG (AU/mL)
S1	0	2
S2	10	10
S3	20	20
S4	50	50
S5	100	100

Controls: Positive and negative controls are prepared in-house and with an assigned range of concentrations. The new lot of positive or negative control should fall into the assigned range.

Controls (AU/mL)	Assigned range
Eu-tTG IgA	
Positive Control	30-75 AU/mL
Negative Control	< 5 AU/mL
Eu-tTG IgG	
Positive Control	35-65
Negative Control	< 6 AU/mL

Stability:

Shelf-life: The shelf life was determined by using 3 lots of the test kit in real time stability studies. The claimed shelf life for the test kits is 12 months when stored at 2-8°C.

Open vial stability: Five calibrators were stored at 2-8°C for two months and then tested. The claimed shelf life stability is 2 months when stored at 2-8°C.

Sample stability: Specimens should be stored at 2-8°C for no longer than five days. For longer storage, serum specimens should be frozen at -20°C.

Repeated freezing and thawing of samples should be avoided.

- d. *Detection limit:*

The analytical sensitivity (detection limit) of the Eu-tTG IgA and Eu-tTG IgG were evaluated in accordance with CLSI Standard EP17-A. The limit of blank (LoB) was determined by running six serum diluent samples (buffer

solution provided with the kit) in ten replicates for a total of 60 measurements. The limit of detection (LoD) was determined and calculated by running six low-level (concentrations around the cutoff) clinical samples in ten replicates each, for a total of 60 measurements.

	LoB (AU/mL)	LoD (AU/mL)
Eu-tTG IgA	0.05	1.6
Eu-tTG IgG	0.02	1.5

e. *Analytical specificity:*

Interference by endogenous substances:

Interference was studied according to EP 7-A2. Five serum samples with known tTG antibody levels were mixed with potentially interfering substances. The study included two positive, one negative and two around the cut-off samples with known concentrations of the anti-tTG antibodies were tested with bilirubin (342 µmol/L), hemoglobin (Hb, 2 g/L), lipid, (triglycerides, 130 mg/dL) and rheumatoid factor (RF, 100 AU/mL). The sponsor states that there is no significant interference up to the levels stated above. The package inserts included a statement stating, “Do not use hemolyzed, hyperlipemic, hemolytic, heat-treated or contaminated serum samples.”

Cross reactivity to other autoantibodies: Cross reactivity was tested using a total of 73 clinical patient samples from individuals with autoimmune disorders, such as: Hashimoto’s Thyroiditis, Graves’ Disease, Antinuclear Antibodies/Systemic Lupus Erythematosus (ANA/SLE) positive and Cyclic Citrullinated Peptide (CCP) positive; patients with IBD and *H. pylori* infection. One (1) CCP positive, two (2) Hashimoto’s Thyroiditis and one (1) Graves’ Disease out of 73 samples (4/73, 5.5%) were tested positive for Eu-tTG IgA. One (1) Hashimoto’s Thyroiditis and two (2) Graves’ Disease out of 73 samples were tested positive with the Eu-tTG IgG (3/73, 4.1%).

Condition	n	Eu-tTG IgA Positive n (%)	Eu-tTG IgG Positive n (%)
IBD*	17	0 (0%)	0 (0%)
CCP Positive	9	1 (11%)	0 (0%)
ANA/SLE Positive	21	0 (0%)	0 (0%)
<i>H. pylori</i> Positive	10	0 (0%)	0 (0%)
Hashimoto's Thyroiditis	8	2 (25%)	1 (12.5%)
Graves' Disease	8	1 (12.5%)	2 (25%)
Total	73	4 (5.5%)	3 (4.1%)

*IBD samples include Ulcerative Colitis & Crohn's Disease samples.

e. *Assay cut-off:*

CLSI C28-A2 was used to perform the cut-off study. The normal range of the assay was established by testing 153 samples, of which 103 are healthy subjects and 50 non-celiac controls (IBD patients), on each assay. The assay cut-off was calculated by the mean plus 3 standard deviations of the detection levels for the tTG IgA and tTG IgG from the healthy subjects, respectively.

The assay cut-off for the Eu-tTG IgA was determined to be 9 AU/mL. When cut-off value is 9 AU/mL, 146 out of 153 negative samples (95.4%) were identified as negative. The remaining 7 samples fell in the range of 9-14.8 AU/mL, and are considered borderline. The assay cut off values of the Eu-tTG IgA was determined as follows:

- <9 AU/mL Negative
- 9-16 AU/mL Borderline
- >16 AU/mL Positive

The assay cut-off for the Eu-tTG IgG was determined to be 20 AU/mL. When using this cut-off value, 150 out of 153 negative samples (98.0%) were identified as negative. The assay cut off value of the Eu-tTG IgG was determined as follows:

- <20 AU/mL Negative
- ≥20 AU/mL Positive

2. Comparison studies:

a. *Method comparison with predicate device:*

The Eu-tTG IgA assay kit was tested in accordance to CLSI EP-A2. For the tTG IgA, these samples consisted of 62 samples from clinically diagnosed (biopsy confirmed) celiac patients and 117 samples from blood donors and other disease/conditions. For tTG IgG, 51 of the 178 samples were from clinically diagnosed celiac patients including 8 samples from IgA deficient patients and 127 samples from blood donors and other disease/conditions. tTG IgA concentrations of the tested samples were within the linear range of the assay. The table

below summarizes the number of serum samples used for the method comparison study for each test:

No. & Origin of Samples	Immunoassay Kit	
	Eu-tTG IgA	Eu-tTG IgG
Positive Celiac Serum Samples	62	51(8 IgA def)
Negative Samples from Blood Donors	57	56
Autoimmune Disease (CCP & ANA)	18	19
Inflammatory Bowel Diseases (IBD)	24	29
Food Intolerance (FI)	10	13
Infectious Disease (<i>H. pylori</i>)	8	10
Total	179	178

Eu-tTG IgA with borderline samples considered positive

		Eu-tTG IgA umana Test (Cut-off = 7 AU/mL)		
		Positive	Negative	Total
Eu-tTG IgA (Cut-off = 9 AU/mL)	Positive	70	2	72
	Negative	4	103	107
	Total	74	105	179

Positive % Agreement = 94.6% (95% CI 86.7% - 98.5%)

Negative % Agreement = 98.1% (95% CI 93.3% - 99.8%)

Overall % Agreement = 96.6% (95% CI 92.8% - 98.8%)

Discrepant Result Analyses – Four samples tested negative with the Eu-tTG IgA were positive with the predicate. Out of these four (4) samples, two were from Inflammatory Bowel Disease (IBD) patients and two from food intolerance patients. Two negative samples (one healthy and one IBD) tested positive with the Eu-tTG IgA but negative with the predicate were around the borderline concentrations, 9 to 16 AU/mL.

Eu-tTG IgA with borderline samples considered negative

		Eu-tTG IgA umana Test (Cut-off = 7 AU/mL)		
		Positive	Negative	Total
Eu-tTG IgA (Cut-off = 16 AU/mL)	Positive	61	0	61
	Negative	13	105	118
	Total	74	117	179

Positive % Agreement = 82.4% (95% CI 71.1% - 90.3%)

Negative % Agreement = 100.0% (95% CI 96.5% - 100.0%)

Overall % Agreement = 92.7% (95% CI 87.9% - 96.1%)

The Eu-tTG IgG test was tested in comparison to the predicate, Aeskulisa tTG G, using 178 clinical samples with analyte concentrations within the linear range of the assay. These samples consisted of 34 samples from

clinically diagnosed celiac patients, 10 total IgA deficient celiac patients, and 147 negative samples, of which 75 were from healthy blood donors, 29 IBD patients, 14 patients affected by food intolerances and 29 clinical samples with autoimmune or infectious diseases:

		Aeskulisa tTG G (Cut-off = 15 AU/mL)		
		Positive	Negative	Total
Eu-tTG IgG Cut-off = 20 AU/mL	Positive	48	7	55
	Negative	6	117	123
	Total	54	124	178

Positive % Agreement = 88.9% (95% CI 77.4% - 95.8%)

Negative % Agreement = 94.4% (94.4% CI 88.7% - 97.7%)

Overall % Agreement = 92.7% (95% CI 87.8% - 96.1%)

b. *Matrix comparison:*

Not applicable

3. Clinical studies:

a. *Clinical Sensitivity and Clinical Specificity:*

The clinical study for Eu-tTG IgA was performed in four sites – three external and one in-house. The study included 363 clinical samples, of which 121 were from celiac patients, 196 healthy subjects and 46 samples from autoimmune disorders, infectious disease and inflammatory bowel disease (IBD) patients. The positive celiac patient samples were from patients diagnosed with clinical findings and/or confirmed with biopsy. The tables below demonstrate the clinical performance of the Eu-tTG IgA assay:

Clinical Performance of Eu-tTG IgA (Eu-tTG IgA with borderline samples considered positive using Cut-off of 9 AU/mL):

		Celiac Disease		Total
		Positive	Negative	
Eu-tTG IgA	Positive	118	4	122
	Negative	3	238	241
	Total	121	242	363

Sensitivity = 97.5% (95% C.I. 92.9% - 99.5%)

Specificity = 98.3% (95% C.I. 95.8% - 99.5%)

Clinical Performance of Eu-tTG IgA (Eu-tTG IgA with borderline samples considered negative using Cut-off of 16 AU/mL)

		Celiac Disease		Total
		Positive	Negative	
Eu-tTG IgA	Positive	103	2	105
	Negative	18	240	258
	Total	121	242	363

Sensitivity = 85.1% (95% C.I. 77.5% - 90.9%)

Specificity = 99.2% (95% C.I. 97.0% - 99.9%)

The clinical study for Eu-tTG IgG was performed in four sites – three external and one in-house. The study included 407 clinical samples, of which 165 were from positive celiac patients, 196 healthy subjects and 46 autoimmune disorders, infectious disease and IBD patient samples. The positive celiac patient samples were from patients diagnosed with clinical findings and confirmed with biopsy.

Clinical Performance of Eu-tTG IgG

		Celiac Disease		Total
		Positive	Negative	
Eu-tTG IgG	Positive	94	14	108
	Negative	71	228	299
	Total	165	242	407

Sensitivity = 57.0% (95% C.I. 49.0% – 64.6%)

Specificity = 94.2% (95% C.I. 90.5% – 96.8%)

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

n/a

4. Clinical cut-off:

See assay cut-off

5. Expected values/Reference range:

The expected value in the normal population is negative. However, the incidence of celiac disease in the normal population is about 1%. Some apparently healthy, asymptomatic individuals may test positive for tTG antibodies.

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.