510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k041174

B. Purpose for Submission:
New device

C. Analyte:
Anti-tissue transglutaminase (tTG) IgA antibody

D. Type of Test:
Qualitative and semi-quantitative, EIA

E. Applicant:
Sweden Diagnostics (Germany) GmbH
Pharmacia Diagnostics AB

F. Proprietary and Established Names:
Celikey® Tissue Transglutaminase (human, recombinant) IgA Antibody

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:
Celikey® tTG (human, recombinant) IgA Antibody Assay is intended for the semiquantitative and qualitative measurement of anti-tissue transglutaminase (tTG) IgA antibodies in human serum and plasma. Celikey is based on recombinant human tissue transglutaminase as antigen and is useful as an aid in the clinical diagnosis of patients with celiac disease.

2. Indication(s) for use:
Same as Intended Use.

3. Special condition for use statement(s):
   The device is for prescription use only.

4. Special instrument Requirements:
   None

I. Device Description:

The assay kit consists of (1) 12 or 6 human recombinant tTG coated microplate strips, (2) horseradish peroxidase conjugated anti-human IgA, (3) TMB substrate, (4) ready-to-use 6 level calibrators (tTG antibody concentrations of 0, 3, 7, 16, 40 and 100 U/mL), (5) ready-to-use positive control, (6) ready-to-use negative control, (7) wash buffer concentrate (20x), (8) sample diluent concentrate (5x) and (9) stop solution. Calibrators, positive and negative controls are diluted human sera.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   INOVA QUANTA Lite™ tTG (Tissue Transglutaminase)

2. Predicate K number(s):
   k982366

3. Comparison with predicate:

DEVICE	PREDICATE
A. Similarities	
Intended Use. For the semiquantitative and qualitative measurement of anti-tissue transglutaminase (tTG) IgA antibodies in human serum and plasma. Celikey is based on recombinant human tissue transglutaminase as antigen and is useful as an aid in the clinical diagnosis of patients with celiac disease.	For the semi-quantitative detection of IgA antibodies to tissue transglutaminase (endomysium) in human serum. Detection of these antibodies is an aid in diagnosis of certain gluten sensitive enteropathies such as celiac disease and dermatitis herpetiformis.
Assay type – ELISA	Same
Analytes – Anti-tTG antibody	Same
Capture antigens – tissue transglutaminase	Same
Conjugate - Horseradish peroxidase	Same
Substrate – TMB	Same
Sample dilution – 1:101	Same
B. Differences	
Assay format – Qualitative and semi-quantitative	Semi-quantitative
Source of tTG – human recombinant from baculovirus/insect cell system	Guinea pig liver
Sample type – Serum and plasma	Serum
Calibrators – 6 levels	None

DEVICE	PREDICATE
A. Similarities	
Controls – positive and negative	Negative, low positive and high positive controls
Cut-off values Semi-quantitative - negative <5 U/mL equivocal 5-8 U/mL positive >8 U/mL Qualitative - negative ratio <1.0 equivocal ratio 1.0-1.4 positive ratio >1.4	Negative <20 units weak positive 20-30 units moderate to strong positive >30 units

K. Standard/Guidance Document Referenced (if applicable):
None referenced.

L. Test Principle:
The Celikey tTG IgA Antibody assay is an indirect noncompetitive enzyme immunoassays. The wells of a microplate are coated with recombinant human tTG antigen. Diluted patient samples are added to the microplate wells and antibodies specific for tTG if present will bind to the immobilized antigen. Unbound samples are washed away and an enzyme labeled second antibody (conjugate) is added to each well and bind to the antigen/antibody complex to form an enzyme labeled conjugate-antibody-antigen complex. After washing away any unbound enzyme conjugate, the chromogenic substrate is added. The enzyme labeled antigen-antibody complex converts the substrate to form a color solution. The rate of color formation is a function of the amount of conjugate complexed with the bound antibody and therefore is proportional to the concentration of the autoantibody in the patient sample.

M. Performance Characteristics (if/when applicable):
1. Analytical performance:
   a. *Precision/Reproducibility:*
      Three serum samples (low, medium, high, equivocal, negative) from a serum bank were diluted 1:101 and assayed in duplicates for 18 runs. Calibrators were also analyzed in duplicates. Specification was % CV<15%. The specifications were met. The table below summarizes the results.

Sample ID	Mean (U/mL)	% CV		
		Within	Between	total
A	29.9	4.5	7.0	8.3
B	16.7	3.2	8.7	9.3
C	4.5	4.4	4.9	6.6

   b. *Linearity/assay reportable range:*
      Dilution study - Three positive samples from a serum bank and Calibrator S6 were used in this study. Depending on the tTG IgG

antibody concentration, each sample was pre-diluted to a specified dilution prior to further dilution with Sample Diluent to 1:1, 2:3, 1:2, 1:4, 1:8, 1:16, and 1:32. Calibrator S6 was diluted to 1:1, 2:3, 1:2, 1:4 and 1:8. Calibrators, Controls and each dilution were measured in duplicates. Specifications were that observed/expected percents should be within ± 20% for at least 3 successive dilutions of each tested sample. The dilutions met the criteria and were considered linear.

Recovery study – Two samples containing 15 U/mL and 46.8 U/mL of tTG IgA from a serum bank were diluted to 1:101 and spiked with 1/10 volume of Calibrator points S1, S4, S5 and S6, i.e. 1.6, 4 and 10 U/mL. The unspiked and spiked samples, the Calibrators and Controls were measured in duplicates. Acceptance criteria were that recovery (%) ± 20% of the expected values. The low concentration sample had percent recoveries ranged from 86% to 98.4% and the high concentration sample from 103.1% to 114.7%. Study results met the acceptance criteria.

Reportable range – 0.02 U/mL to 100 U/mL

c. *Traceability (controls, calibrators, or method):*
There is no recognized reference material for tTG autoantibodies. Results are reported in arbitrary units

d. *Detection limit (functional sensitivity):*
Sample Diluent was diluted according to Directions for Use and measured 56 times on one plate. Calibrators and Controls were analyzed in quadruplicates. Analytical sensitivity was calculated as the mean of the optical densities (OD) of the Sample Diluent plus 3SD and expressed in U/mL. The discrimination value D for differentiating the lowest calibrator point and the background was calculated using the following equation:

$$D = \frac{\eta_B - \eta_A}{\sqrt{(\sigma_B^2 - \sigma_A^2)}}$$

where A = Sample Buffer; B = Calibrator S2; $\acute{\eta}_A$, $\acute{\eta}_B$ = mean OD; σ_A, σ_B = SD.

Acceptance criteria specified that the (mean OD + 3SD) of the Sample Diluent < Calibrator point S2, detection limit ≤1 U/mL, and the discrimination value D >2.0. The (mean +3SD) was 0.012 OD for the Sample Diluent, which corresponded to analytical sensitivity of 0.02 U/mL. The other criteria were also met. The lower limit of the measuring range was set to <0.02 U/mL.

e. *Analytical specificity:*

Interference was tested against potentially interfering substances found in blood: bilirubin, hemoglobin, chyle, and rheumatoid factor. A sample with known tTG IgA concentration from a serum bank was diluted 1:101 and spiked with buffer or different amounts of interfering substances. The spiked and unspiked samples were analyzed in triplicates. The Calibrators and Controls were analyzed in duplicates. Acceptance criteria were that spiked samples should be ≤20% variation from unspiked sample. The concentrations of the spiked-in substances are shown in table below. All samples met acceptance criteria and showed no significant interference on the test results.

Additives		Final sample concentration			
	Blank	II	III	IV	V
Bilirubin F (mg/dL)	0.0	4.8	9.5	13.7	18.2
Bilirubin C (mg/dL)	0.0	5.5	11.0	16.5	22.0
Chyle (Units)	0.0	700	1400	2100	2800
Hemoglobin (mg/dL)	0.0	122.5	245.0	367.5	490.0
RF (IU/mL)	0.0	104.0	312.0	520.0	n.a.-

Additives	Mean tTG IgA conc. (U/mL)	Recovery (%)			
		II	III	IV	V
Bilirubin F	36.7	105.6	108.1	116.2	118.0
Bilirubin C	37.4	102.6	109.6	106.8	116.9
Chyle	36.2	104.0	109.6	106.8	119.9
Hemoglobin	39.6	103.4	107.5	112.2	101.3
RF	44.9	101.3	103.4	105.4	n.a.-

Two additional samples (1 negative and 1 equivocal) were added to the interference study because the original sample had high tTG IgA concentration and might not be affected by the amount of interfering substances spiked-in. No significant interference was observed.

Crossreactivity was assessed by testing 60 sera positive for other antibodies including ANA, SS-A, SS-B, TPO, dsDNA Cardiolipin IgG, PR3, GBM, MPO, RF, PC, LKM1, actin and HCV. Fifty sera were from an external source and 10 sera were ANA human reference sera from CDC. None of the serum samples was found positive. Two samples were in the equivocal range with one from a SLE patient and the other the CDC reference serum for Sm.

f. *Assay cut-off:*

The semi-quantitative cut-points were determined by measuring 1051 samples from apparently healthy blood donors, equally distributed by sex and age from a serum bank. Diluted samples and standards were analyzed in duplicates. Controls were analyzed in

quadruplicates. Specifications for the study were the mean +2SD <lower limit of the equivocal range, and the mean +3SD <upper limit of the equivocal range. Results showed that there was no difference between gender and age. The mean and median concentration of tTG IgA antibodies were 0.6 U/mL and 0.4 U/mL respectively. The mean+2SD was 2.3 U/mL, the mean+3SD was 3.2 U/mL and the 95 percentile was 1.9 U/mL. Based on these results, the following values were selected for negative, equivocal, and positive:

<5 U/mL = negative
5-8 U/mL = equivocal
>8 U/mL = positive

2. Comparison studies:

a. *Method comparison with predicate device:*
Ninety-seven clinically defined patient samples and 20 normal samples were tested on the new device, the predicate device and an IFA anti-endomysial (EMA) assay. The patient samples consisted of 65 celiac disease (CD), 10 suspected CD, 13 inflammatory bowel disease (IBD), 4 Morbus Crohn/Colitis Ulcerosa (Crohn/UC), 2 questionable CD, 1 gluten free diet (GFD), 1 CD/GFD and 1 diagnosis unknown. Of the 65 CD samples, 7 were EAM negative. Of the 20 healthy donor samples, three were EAM positive. One of the four Crohn/UC samples was also EAM positive. The Celikey and INOVA tTG IgA results of the different cohorts are summarized in the following two tables.

Celikey® tTG IgA (U/mL)	Cohorts				
	CD	Susp Crohn/UC	Crohn/UC	IBD	Healthy
N	65	10	4	13	20
Mean±SD	45.94±32.16	2.15±1.35	25.0±47.15	2.92±0.74	7.42±15.23
Median	35.0	2.2	1.7	2.8	2.5
Range	0.1 to >100	0.3 to 1.46	0.8 to 95.7	1.1to 4.9	0.2 to 60.9

INOVA tTG IgA (U/mL)	Cohorts				
	CD	Susp Crohn/UC	Crohn/UC	IBD	Healthy
N	65	10	4	13	20
Mean±SD	115.49±104.65	8.51±2.94	87.0±161.65	6.87±1.97	23.0±41.33
Median	80.74	8.18	6.92	6.94	7.83
Range	2.38 to 433	3.89 to 12.88	4.7 to 329.5	4.2 to 11.28	3.65 to 140.6

The predicate comparative results are as follows:

		INOVA Quanta Lite tTG IgA		
		+	-	Total
Celikey tTG IgA	+	61	8	69
	-	0	46	47
	Equiv	1	1	2
	Total	62	55	117

% positive agreement = 98.4% (61/62) (95% CI: 95.3% to 100%)
% negative agreement = 85.2% (46/54) (95% CI: 75.7% to 94.7%)
% total agreement = 91.5% (107/117), (95% CI: 86.5% to 96.6%)

The Celikey^{equ}/Quanta Lite^{pos} sample was from an EMA positive sample from a CD patient whereas the Celikey^{equ}/Quanta Lite^{neg} sample was EMA negative and from a possible CD patient. Six of the eight Celikey^{pos}/Quanta Lite^{neg} discrepant samples were from CD patients. One of the remaining two samples was from a CD patient on gluten-free diet (CD/GFD) and the other from a normal control. Three of the six CD discrepant samples were EAM positive. The CD/GFD sample was also EMA positive. The overall agreement between the Celikey and the Quanta Lite tTG assays was 91.5%.

b. *Matrix comparison:*
   The device uses both serum and plasma samples. To demonstrate that the new assay gives the same results for serum, heparin plasma, citrate plasma and EDTA plasma from the same patient, 10 tTG IgA antibody negative samples (tTG IgA concentrations ranged from 0.1 U/mL to 1.5 U/mL) for each matrix were spiked with 10 different tTG IgA positive sera (tTG IgA concentrations ranged from 20.4 U/mL to 43.3 U/mL). The negative and the spiked samples were run in quadruplicates and the calibrators and controls in duplicates. Acceptance criteria for this study were that the percent deviation between serum and plasma results for positive samples should not be greater than ± 20% and negative samples should be negative for both serum and plasma matrices. The data showed no differences greater than ± 20% with deviations ranged from -14.9% to 19.6% for citrate, -15.4% to -5.4% for EDTA plasma and 4.7% to 19.2% for heparin. No negative sample changed from negative to positive. Thus the specifications were met.

   The matrix comparison study was expanded using serum and plasma samples from two additional patients (one negative and one equivocal) sample. Results were within the acceptance criteria.

3. Clinical studies:
   a. *Clinical sensitivity:*
      One hundred and fifty two clinically characterized samples were obtained from hospitals or laboratories and consisted of 73 CD, 17 Crohn/UC, 19 IBD and 43 normal controls. These samples were analyzed using the Celikey$^{®}$ tTG IgA Antibody Assay and the EMA IIF assay. The following table summarizes the age distribution and tTG IgA results of the 4 cohorts.

	Age (years)		tTG IgA (U/mL)	
	Mean ± SD	Range	Mean ± SD	Range
CD	30.2 ± 19.1	4 to 74	57.89 ± 35.7	6.2 to >100
Crohn/UC	42.8 ± 9.9	22 to 57	0.59 ± 0.60	0.1 to 2.3
IBD	11.4 ± 3.3	3 to 16	2.16 ± 1.16	0.2 to 3.9
Healthy	37.6 ± 22.8	1 to 71	1.13 ± 1.67	0.1 to 10

In the disease cohort, three subjects had equivocal tTG IgA results which were EMA positive. In the normal cohort, one subject had a low positive tTG IgA result (10 U/mL) but was EMA negative. Based on this study, the clinical sensitivity of the Celikey tTG IgA assay was therefore determined to be 95.9% (95% CI: 91.4% to 100%) (see table below).

		Celiac disease		
		Positive	Negative	Total
Celikey IgA	Positive	70	1	71
	Equivocal	3	0	3
	Negative	0	78	78
	Total	73	79	152

   b. Clinical specificity:
   The clinical specificity of the Celikey tTG IgA assay as determined by the study described in 3(a) was 98.7% (95% CI: 96.2% to 100%)

4. Clinical cut-off:
   Same as assay cut-off.

5. Expected values/Reference range:
   Expected value in the normal population is negative. The frequency distribution for tTG IgA antibodies as determined by the Celikey tTG IgA assay on the 152 clinically defined samples is summarized below (equivocal samples excluded).

	N	#Positive	Frequency
CD	73	70	95.8%
IBD	19	0	0%
Crohn/UC	17	0	0%
Healthy	43	1	2.3%

N. Conclusion:
   The submitted information in this premarket notification is complete to support a substantial equivalence decision.