

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION

A. 510(k) Number:

k072967

B. Purpose for Submission:

New device

C. Measurand:

Anti-F-actin IgA antibody

D. Type of Test:

Quantitative enzyme-linked immunosorbent assay (ELISA)

E. Applicant:

INOVA Diagnostics, Inc.

F. Proprietary and Established Names:

QUANTA Lite™ F-actin IgA ELISA

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
MVM	Class II	21 CFR 866.5660 Multiple autoantibodies immunological test system	Immunology 82

H. Intended Use:

1. Intended use(s):

The QUANTA Lite F-actin IgA Assay is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of IgA antibodies to F-actin component of smooth muscle in human serum. In patients with clinical findings consistent with celiac disease, the presence of anti-F-actin IgA antibodies may assist in estimating the likelihood of significant intestinal villous atrophy.

2. Indication(s) for use:

Same as the intended use

3. Special conditions for use statement(s):

Prescription use only

4. Special instrument requirements:

ELISA plate reader, plate shaker or rotator

I. Device Description:

The device consists of: polystyrene microwell ELISA plate coated with purified F-actin antigen (12 – 1x8 wells), Horseradish peroxidase (HRP) goat anti-human IgA conjugate, TMB chromogen, F-actin IgA ELISA Low and High Positive controls (human sera), negative control (human serum), HRP sample diluent, HRP wash concentrate (40x) and stop solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):

QUANTA Lite™ h-tTG IgA

2. Predicate 510(k) number(s):
k011566
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	QUANTA Lite F-actin IgA	QUANTA Lite h-tTG IgA
Test principle	Enzyme-linked immunosorbent assay	Same
Assay format	Semi-quantitative	Same
Platform	96-well microtiter plate	Same
Negative control	Prediluted human serum, ready-to-use	Same
Sample type and dilution	Serum at 1:101	Same
Sample volume	5 µL	Same
Enzyme conjugate	HRP goat anti-human IgA	Same
Substrate	TMB chromogen	Same
Incubation times	30-30-30 minutes	Same
O.D. reading	450 nm (and 620 for dual wavelength readings)	Same

Differences		
Item	Device	Predicate
Intended use	Semi-quantitative detection of F-actin IgA	Semi-quantitative detection of h-tTG IgA
Indications for use	To aid in the assessment of intestinal damage in patients with celiac disease	To aid in the diagnosis of certain gluten sensitive enteropathies such as celiac disease and dermatitis herpetiformis
Capture antigen	Purified F-actin antigen	Purified native human tissue transglutaminase
High Positive and Low Positive Controls	Prediluted, ready-to-use F-actin IgA	Pre-diluted, ready-to-use h-tTG IgA
Cut-off (units)	25.0	20

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

None referenced.

L. Test Principle:

Purified F-actin antigen is bound to the wells of a polystyrene microwell plate under conditions that will preserve the antigen in its native state. Pre-diluted controls and diluted patient sera are added to separate wells, allowing any F-actin antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgA conjugate is added to each well. A second

incubation allows the enzyme labeled anti-human IgA to bind to any patient antibodies, which have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgA, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops. The assay can be evaluated spectrophotometrically by measuring and comparing the color intensity that develops in the patient wells with the color in the control wells.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Intra-assay

To assess intra-assay precision, 6 samples containing a range of F-actin antibody levels (14, 17.4, 37, 38.8, 104.2 and 152 units) were assayed in 7 replicates each. Results showed acceptable % CV ranging from 0.9% to 4.6%.

Inter-assay

To assess inter-assay precision, 6 samples comprised of 2 negatives, 2 moderates, 2 high positives and the high positive control were assayed in duplicate, twice daily (once in the morning and once in the afternoon) for 3 days. Results showed acceptable %CV ranging from 3.7% to 6.4%.

b. *Linearity/assay reportable range:*

No claims for linearity. Results are reported as follows:

Negative – 0.0-20.0 units

Equivocal – 20.1-24.9 units

Positive \geq 25 units

Samples with OD reading above the readable range of the plate reader can be serially diluted and re-run or reported as greater than the highest measurable OD.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

There is no reference standard for F-actin IgA antibodies. Arbitrary values are assigned to the positive and negative controls.

d. *Detection limit:*

Not applicable.

e. *Analytical specificity:*

No data provided due to endogenous substances such as hemoglobin, bilirubin and lipids. The package insert states that grossly hemolyzed, icteric, microbially contaminated, or heat-treated samples should be avoided.

Cross-reactivity

Initially sera from 36 patients with autoimmune/infectious disease antibodies consisted of gastric parietal cell (1), RNP (2), SSA (1), SSB (2), Scl-70 (1), Jo-1 (2), glomerular basement membrane (3), LKM-1 (3), soluble liver antigen (2), centromere (3), chromatin (1), autoimmune hepatitis (3), primary biliary cirrhosis (PBC) (3), Hepatitis B (HBV) (3), Hepatitis C (HCV) (3) and

primary sclerosing cholangitis (PSC) (3) were tested for cross-reactivity with the QUANTA Lite F-actin IgA ELISA. Since positive results were found with several autoimmune and viral liver disease specimens, additional specimens from patients with liver disease were tested. F-actin IgA were positive in 60% (12/20) autoimmune hepatitis, 24% (5/21) PBC, 75% (15/20) PSC, 37.5% (3/8) HCV and 75% (6/8) HBV samples. Of the 38 F-actin IgA positive samples, 78.9% (30/38) were also positive for F-actin IgG. A limitation is included in the package insert which states “Detection of F-actin IgA in patients with liver disease should be interpreted with caution since the presence of F-actin IgG antibodies in this disease group is often accompanied by F-actin IgA which may be unrelated to intestinal pathology. F-actin IgA antibodies should be measured only in patients with celiac disease as an aid to assessment of intestinal damage”.

f. Assay cut-off:

The cut-off value was initially established using 500 serum samples from apparently healthy controls (250 male and 250 female) with median age of 41y (range 18-80y). These samples were also screened with the h-tTG IgA assay and found to be positive in 7 samples (1.4%). Using the arbitrary cut-off value of 25 units for F-actin IgA, 461 samples were below the cut-off (92.2%). Of the 39 anti-F-actin IgA positive samples, three were found to be also positive for h-tTG IgA antibodies and one of the three was also positive for endomysial antibody. Since these samples were also tTG positive, it is likely that they may be from undiagnosed celiac patients. To validate the appropriateness of the cut-off, the sponsor tested sera from 196 celiac patients and 484 non-celiac patients. Results showed that 38.8% (76/196) of the celiac patient samples were above 25 units and 94.2% (456/484) of the non-celiac patient samples were below 25 units.

2. Comparison studies:

a. Method comparison with predicate device:

The Marsh criteria were used to measure the degree of intestinal damage and defined as: Marsh 3a score (mild villous atrophy), Marsh 3b score (marked villous atrophy) and Marsh 3c score (total villous atrophy). For this comparison study, 157 celiac patients consisted of 20 Marsh 3a, 36 Marsh 3b and 101 Marsh 3c were tested by both F-actin IgA and h-tTG IgA ELISA assays. Results showed agreement between the two tests increased with increased atrophy. The tables below summarize results for each Marsh score and all patients combined.

Marsh 3a score		h-tTG IgA ELISA		
		+	-	Total
F-actin IgA ELISA	+	7	1	8
	-	8	4	12
	Total	15	5	20

Positive Percent Agreement: (7/15) = 46.7%

Negative Percent Agreement: (4/5) = 80.0%

Overall Agreement: (11/20) = 55%

Marsh 3b score		h-tTG IgA ELISA		
		+	-	Total
F-actin IgA ELISA	+	7	1	8
	-	8	20	28
	Total	15	21	36

Positive Percent Agreement: (7/15) = 46.7%

Negative Percent Agreement: (20/21) = 95.2%

Overall Agreement: (27/36) = 75%

Marsh 3c score		h-tTG IgA ELISA		
		+	-	Total
F-actin IgA ELISA	+	60	0	60
	-	38	3	41
	Total	98	3	101

Positive Percent Agreement: (60/98) = 61.2%

Negative Percent Agreement: (3/3) = 100%

Overall Agreement: (63/101) = 62.4%

All groups combined		h-tTG IgA ELISA		
		+	-	Total
F-actin IgA ELISA	+	74	2	76
	-	54	27	81
	Total	128	29	157

Positive Percent Agreement: (74/128) = 57.8%

Negative Percent Agreement: (27/29) = 93.1%

Overall Agreement: (101/157) = 64.3%

b. Matrix comparison:

Both assays use serum.

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

For this study, 445 patient samples collected from 1 US and 3 non-US clinical sites were tested with the QUANTA Lite F-actin IgA and h-tTG IgA ELISA assays. All samples had biopsy results and Marsh scores. The samples were blinded and testing was performed by the manufacturer. The following table summarizes F-actin IgA and tTG IgA results as stratified according to the Marsh scores. Of the 445 samples, 19.3% (86/445) were F-actin IgA positive as compared to 35% for tTG IgA. Of the F-actin IgA positive samples 82.8% (72/86) were Marsh 3 and 69.9% if the 86 were Marsh 3c. Results supported the intended use claim that F-actin IgA positivity correlated with patients with more severe intestinal atrophy especially patients classified as Marsh3c, i.e. total villous atrophy.

	Marsh Score					
	0	1	2	3a	3b	3c
#Patients	128	143	17	20	36	101
Median F-actin IgA (units)	7.2	9.0	8.9	20.2	10.4	37
Mean F-actin IgA (units)	11.1	10.9	11.3	42.4	17.8	64.6
#F-actin IgA positive	5	8	1	4	8	60
%F-actin IgA positive	3.8	3.5	5.9	20	22.2	59.4
#tTG IgA positive	3	14	2	15	28	94
%tTG IgA positive	2.3	9.8	11.8	75	77.8	93.1

The F-actin IgA results were also analyzed based on the presence of absence of Marsh 3 histology (see below). Sensitivity was shown to be 48.4% (95% CI, 40.4% to 56.5%) and specificity 95.1% (95% CI, 92.0% to 97.3%).

		Marsh Score		
		3a+3b+3c	0+1+2	Total
F-actin IgA ELISA	+	76	14	90
	-	81	274	355
	Total	157	288	445

If results were evaluated for Marsh 3c alone, sensitivity and specificity were 59.4% (95% CI, 49.2% to 69.1%) and 91.3% (95% CI, 87.7% to 94.0%) respectively.

		Marsh Score		
		M3c	Non M3c	Total
F-actin IgA ELISA	+	60	30	90
	-	41	314	355
	Total	101	344	445

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

Not applicable.

4. Clinical cut-off:

See assay cut-off

5. Expected values/Reference range:

The expected value for the normal population is ≤ 25 units.

N. Proposed Labeling:

The labeling is sufficient and 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.