

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY

A. 510(k) Number:

k061841

B. Purpose for Submission:

New device

C. Measurand:

Intrinsic factor antibodies

D. Type of Test:

Semi-quantitative Enzyme-linked immunosorbent assay (ELISA)

E. Applicant:

INOVA Diagnostics, Inc.

F. Proprietary and Established Names:

QUANTA Lite™ Intrinsic Factor Antibody ELISA

G. Regulatory Information:

1. Regulation section:
CFR 862.1810 Vitamin B₁₂ test system
2. Classification:
Class II
3. Product code:
LIG Radioassay, intrinsic factor blocking antibody
4. Panel:
CH 75

H. Intended Use:

1. Intended use:
QUANTA Lite™ Intrinsic Factor Antibody ELISA is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of Intrinsic Factor antibodies of the IgG class in human serum. The presence of Intrinsic Factor antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of pernicious anemia.
2. Indication(s) for use:
Same as intended use
3. Special conditions for use statement(s):
For prescription use only
4. Special instrument requirements:
Microwell plate reader capable of measuring OD at 450nm (and 620nm for dual wavelength readings)

I. Device Description:

The QUANTA Lite Intrinsic Factor Antibody ELISA consists of a polystyrene microwell ELISA plate coated with recombinant human intrinsic factor antigen; ELISA negative, low positive and high positive controls; sample diluent; wash concentrate; goat anti-human IgG horseradish peroxidase conjugate; TMB chromogen; and stop solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):
INOVA QUANTA Lite Gastric Parietal Cell Antibody ELISA
2. Predicate 510(k) number(s):
k010558
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	Intrinsic Factor Antibody ELISA	Gastric Parietal Cell Antibody ELISA
Indications for Use	To aid in the diagnosis of pernicious anemia	To aid in the diagnosis of pernicious anemia
Method	ELISA	Same
Calculation of results	Compared to a cut-off control	Same
Result interpretation	≤ 20 U/mL = negative, 20.1-24.9 = equivocal, ≥ 25 = positive	Same
Units	Arbitrary ELISA units	Same
Test matrix	Serum	Same
Solid phase	Coated polystyrene microwell plates	Same
Sample diluent	Tris-buffered saline, Tween 20, absorbents and protein stabilizers	Same
Wash concentrate	Tris-buffered saline and Tween 20	Same
HRP IgG conjugate	Goat anti-human IgG	Same
Controls	Negative, low positive, and high positive	Same

Differences		
Item	Device	Predicate
	Intrinsic Factor antibody ELISA	Gastric Parietal Cell Antibody ELISA
Analyte measured	Anti-intrinsic factor antibodies	Anti-gastric parietal cell antibodies
Capture antigen	Recombinant human intrinsic factor	Purified H ⁺ /K ⁺ ATPase isolated from pig gastric mucosa

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

None referenced

L. Test Principle:

The assay utilizes plastic microwells as a solid phase for attachment of recombinant human intrinsic factor. Pre-diluted controls and diluted patient sera are added to separate wells, allowing any intrinsic factor antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled goat anti-human IgG antibody is added to each well. A second incubation allows the enzyme labeled anti-human IgG to bind to any patient antibodies which have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgG, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops. The assay is evaluated by spectrophotometrically measuring and comparing the color intensity that develops in the patient wells with the color in the control wells. Results determined with the assay are interpreted as negative, equivocal, or positive and are reported in arbitrary units.

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

Intra-assay performance for the assay was evaluated by testing 7 specimens a total of 5 times each. The samples tested ranged from 10.6 to 115.2 Units with %CVs ranging from 1.8 to 8.0%.

	A (HPC)	B	C	D	E	F	G	H
Mean units	108.1	45.4	115.2	108.7	21.3	17.1	10.6	13.4
SD	2.1	0.8	2.5	3.4	1.7	0.8	0.4	0.7
CV%	1.9	1.8	2.2	3.2	8.0	5.0	4.0	5.3

Inter-assay variation was assessed by testing, in duplicate, a panel of 5 specimens including 1 negative, 1 low positive, 1 moderate, 2 high positive and the kit controls twice daily (once in the morning and once in the afternoon) for 3 days. Percent CVs ranged from 3.6 to 14.4 Units.

	HPC	NC	Spec. 1	Spec. 2	Spec. 3	Spec. 4	Spec. 5
Mean units	109.1	1.1	107.9	47.4	13.9	108.0	29.2
SD	5.96	0.16	4.82	1.71	0.69	5.45	2.41
CV%	5.5	14.4	4.5	3.6	5.0	5.0	8.2

b. *Linearity/assay reportable range:*

No claims were made regarding linearity for the assay. It is a semi-quantitative assay with results reported out as negative: 0.0 – 20.0 Units, positive as ≥ 25 Units, or equivocal: 20.1 – 24.9 Units when results are interpreted by comparison to the low positive control value of 25 Units. Specimens giving OD readings above the readable range of the plate reader

may be reported as greater than the highest measurable OD. Alternatively, samples may be serially diluted, re-run and a calculated value obtained. However, reactivity is related to the quantity of antibody present in a non-linear fashion.

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
There is no recognized standard or reference material for anti-intrinsic factor antibodies.

Accelerated stability studies were conducted on 3 lots of antigen coated plates and 3 lots of low and high positive kit controls. Data supported a shelf life of one year. Real time stability studies are currently being performed.

- d. *Detection limit:*
The determination of detection limit/analytical sensitivity was not performed or relevant for this assay.

- e. *Analytical specificity:*
Microbially contaminated, heat-treated, samples with visible particulate grossly hemolyzed or lipemic specimens should not be used in the assay.

- f. *Assay cut-off:*
A panel of 476 asymptomatic, healthy individuals was tested. Ages ranged from 14-78 (median 43). Of the 276 specimens with age and sex information available, 150 were from males and 126 from females. Of the 476 specimens, one was a strong positive at 103.2 Units, one was borderline positive at 25.5 and one was equivocal. Excluding the equivocal result, the specificity of the assay was 99.6%. Excluding the one strong positive result, the mean was 5.7 Units and the median value was 5.0 Units.

2. Comparison studies:

- a. *Method comparison with predicate device:*
Testing was performed on 278 specimens that included 69 specimens from healthy individuals and 209 specimens submitted to 2 reference laboratories for gastric parietal cell antibody (GPA) and/or intrinsic factor antibody testing.

QUANTA Lite Intrinsic Factor Antibody ELISA	QUANTA Lite GPA ELISA			
	Positive	Equivocal*	Negative	Total
Positive	22	0	5	27
Equivocal*	0	0	0	0
Negative	52	6	193	251
Total	74	6	198	278

*Equivocal results were considered negative in the calculations

Positive percent agreement: $22/74 = 29.7\%$

Negative percent agreement: $199/205 = 97.1\%$

Overall agreement: $221/278 = 79.5\%$

The agreement between the assays was low because 1) the assays measure different analytes; and 2) not all pernicious anemia (PA) patients have both antibodies. Gastric parietal cell antibodies present earlier in the progression of chronic atrophic gastritis to pernicious anemia and are found in approximately 90% of PA patients. Intrinsic factor antibodies, while specific for PA, are present in only 50-60% of PA patients.

The company also compared to another legally marketed device, the DPC IF bAb [^{57}Co] Radioimmunoassay (k832726). One hundred seventy four specimens submitted to a clinical reference laboratory for IF antibody evaluation were tested. Additional clinical information about these subjects was not available.

QUANTA Lite Intrinsic Factor Antibody ELISA	DPC RIA for Intrinsic Factor Antibody		
	Positive	Negative	Total
Positive	65	0	65
Negative	56	53*	109
Total	121	53	174

*2 equivocal results with the ELISA assay were considered negative in the calculations

Positive percent agreement: $65/121 = 53.7\%$

Negative percent agreement: $53/53 = 100\%$

Overall agreement: $118/174 = 67.8\%$

There is a distinct possibility of interference from therapeutic doses of vitamin B₁₂ in the RIA assay.

b. Matrix comparison:

Both assays use serum as the test matrix.

3. Clinical studies:

a. Clinical Sensitivity:

Clinical sensitivity was established by testing sera from 177 subjects which included 43 with confirmed PA, 55 with presumed PA and 79 suspected PA. In the 43 confirmed PA, the assay interpreted 95.3% (41/43) as positive.

Clinical Status	N =	Intrinsic factor ELISA positive	Intrinsic factor ELISA equivocal	Intrinsic factor ELISA negative
Confirmed PA	43	41	1	1
Presumed PA	55	54	1	0
Suspected PA	79	0	0	79
Total	177	92	2	1

The definitions for the categories are:

Confirmed Pernicious Anemia: laboratory measures consistent with pernicious anemia including gastric parietal cell antibody positive, low vitamin B₁₂, mean corpuscular volume (MCV) increased (typical of pernicious anemia), hematological confirmation of megaloblastic anemia

Presumed Pernicious Anemia: laboratory measures consistent with pernicious anemia including gastric parietal cell antibody positive, low vitamin B₁₂, mean corpuscular volume (MCV) increased (typical of pernicious anemia)

Suspected Pernicious Anemia: one or more tests positive: gastric parietal cell antibody, low vitamin B₁₂, but intrinsic factor antibody assay results were discrepant

b. Clinical specificity:

A total of 499 sera from patients with other autoimmune or infectious disease (n=23), and normal subjects (n=476) were tested to assess potential cross-reactivity of other disease sera with the assay.

Other diseases and normal subjects	N =	IF ELISA positive	IF ELISA equivocal	IF ELISA negative	Total
Other (H. pylori, mitochondrial M2, cytomegalovirus, herpes simplex virus, ASCA, RNP, SSA, SSB, Scl-70, DNA, tissue transglutaminase, glomerular basement membrane)	23	0	0	23	23
Normal	476	2	1	473	476
Total		2	1	496	499

* Sm (1), RNP (1), SSB (1), Histone (3), Scl-70 (1), ribosome P (1), chromatin (2), centromere (1), ASCA (2), GBM (2), Jo-1 (1)

Clinical sensitivity and specificity calculations:

Groups	N
Confirmed pernicious anemia	43
Presumed pernicious anemia	55
Suspected pernicious anemia	79
Disease controls	23
Healthy controls	476
Total	676

Suspected PA included as disease *positive*

	Pernicious anemia		
IF antibody result	+	-	Total
+	92	2	94
-	85	497	582
Total	177	499	676

Clinical sensitivity: $92/177 = 52\%$

Clinical specificity: $497/499 = 99.6\%$

Suspected PA included as disease *negative*

	Pernicious anemia		
IF antibody result	+	-	Total
+	92	2	94
-	6	576	582
Total	98	578	676

Clinical sensitivity: $92/98 = 93.9\%$

Clinical specificity: $576/578 = 99.6\%$

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

Not applicable

4. Clinical cut-off:

See assay cut-off and expected values.

5. Expected values/Reference range:

The expected result in the normal population is negative (≥ 20 Units).

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.