510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k071346

B. Purpose for Submission:
New device

C. Measurand:
Intrinsic factor autoantibodies

D. Type of Test:
Qualitative or semi-quantitative enzyme-linked immunosorbent assay (ELISA)

E. Applicant:
IMMCO Diagnostics

F. Proprietary and Established Names:
ImmuLisa™ Anti-Intrinsic Factor (IF) Antibody ELISA

G. Regulatory Information:

1. Regulation section:
   CFR 862.1810 Vitamin B_{12} test system
2. Classification:
   Class II
3. Product code:
   LIG Radioassay, intrinsic factor blocking antibody
4. Panel:
   Chemistry 75

H. Intended Use:

1. Intended use:
   The ImmuLisa™ Anti-Intrinsic Factor (IF) Antibody ELISA is an enzyme-linked immunosorbent assay (ELISA) for the qualitative and semi-quantitative detection of antibodies to intrinsic factor in human serum 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 405nm (and 620nm for dual wavelength readings)

I. Device Description:
The ImmuLisa Anti-Intrinsic Factor Antibody ELISA consists of microplate well strips coated with recombinant glycosylated human intrinsic factor antigen; positive and negative controls; 4 calibrators; alkaline phosphatase goat anti-human IgG conjugate; serum diluent; pNPP enzyme substrate; stop solution; powdered wash buffer; and a frame holder.

J. Substantial Equivalence Information:

1. Predicate device name(s):

DPC Intrinsic Factor Blocking Antibody RIA Kit

2. Predicate 510(k) number(s):
   k832726
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	ImmuLisa Anti-Intrinsic Factor Antibody ELISA	**DPC Intrinsic Factor Blocking Antibody RIA**
Indications for Use	To aid in the diagnosis of pernicious anemia	To aid in the diagnosis of pernicious anemia
Analyte measured	Anti-intrinsic factor antibodies (Type I and II)	Anti-intrinsic blocking factor antibodies (Type I)
Test matrix	Serum (diluted 1:101)	Serum (undiluted)

Differences		
Item	Device	Predicate
Method	Enzyme-linked immunosorbent assay	Radioimmunoassay
Calculation of results	Compared to a cut-off control	Ratio: negative reference average CPM/average CPM
Solid phase	Antigen coated polystyrene microwell plates	Antigen immobilized on microcrystalline cellulose particles
Calibrators	4 levels	Not applicable
Units	Arbitrary ELISA units	Not applicable
Controls	Negative and positive	Negative, positive and high positive
Label/detector	Alkaline phosphatase labeled goat anti-human IgG	^{57}Cobalt-labeled vitamin B12
Substrate	pNPP	Not applicable
Capture antigen	Recombinant human intrinsic factor	Purified intrinsic factor
Result interpretation	negative = <20 U/mL indeterminent = 20-25 positive = >25	Ratios: negative = <negative cut-off + 0.10 indeterminent = >1.10 and <positive ratio – 0.10 positive = ≥ positive cut-off
Signal detector	Spectrophotometer (absorbance)	Gamma counter (^{57}Cobalt)

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

None referenced

L. Test Principle:

The test is performed as a solid phase immunoassay. Controls, calibrator(s) and patient sera are incubated in the antigen coated microwells to allow specific antibodies present in the serum to bind to the intrinsic factor antigen. Unbound antibodies and other serum proteins are removed by washing the microwells. Bound antibodies are detected by adding an enzyme labeled anti-human IgG conjugate to the microwells. Unbound conjugate is removed by washing. Specific enzyme substrate (pNPP) is then added to the wells and the presence of antibodies is detected by a color change produced by the conversion of pNPP substrate to a colored reaction product. The reaction is stopped and the intensity of the color change, which is proportional to the concentration of antibody, is read by a spectrophotometer at 405 nm. Specimen optical density is compared with optical density of a calibrator (qualitative result reported as positive or negative) or a four point standard curve (semi-quantitative expressed in ELISA units).

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

   Intra-assay performance was based on 16 replicates of 5 specimens ranging from 22 to 150 EU/mL with %CV ranging from 1.4 to 12.3%.

	1	2	3	4	5
Mean EU/mL	22	29	49	87	150
%CV	12.3	4.8	3.2	2.6	1.4

   Inter-assay performance for the assay was evaluated by testing 5 specimens, by 5 operators for 3 days. The samples tested ranged from 22 to 150 Units with %CVs ranging from 5.6 to 13.9%.

	1	2	3	4	5
Mean EU/mL	22	29	49	87	150
%CV	13.9	9.9	9.6	7.9	5.6

   b. Linearity/assay reportable range:

   To determine acceptable linearity, plates were assayed with a positive serum diluted in doubling dilutions from 1:800 to 1:6400. The r-squared values of the resulting standard curves were determined for multiple assays (3 operators, 3 days, 3 samples). An r-squared value greater than 0.95 was considered acceptable. The average r-squared value for the assays was 0.988 and the values ranged from 0.935 – 1.0.

   Dilution recovery:

   For each of 5 tests, two samples of equal volume with known intrinsic factor antibody levels were mixed. Antibody concentration for the mixture was expected to be the average of the antibody concentration of the discrete samples. The antibody levels of the mixed samples were determined and used

to calculate percent recovery. The following results were obtained:

	Mixture	Expected (EU/mL)	Obtained (EU/mL)	Recovery
1	23 EU+28 EU/mL	25.5	23.2	91%
2	23 EU+54 EU/mL	38.5	33.8	88%
3	28 EU+60 EU/mL	44	46.3	105%
4	54 EU+81 EU/mL	67.5	60.4	89%
5	81 EU+137 EU/mL	109	101.5	94%

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 assay components. Data supported a shelf life of 15 months.

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

e. *Analytical specificity:*
Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of the test and should not be used.

f. *Assay cut-off:*
The cut-off value was originally determined by testing 40 adult normal blood donors. In this study, the mean of the normal subjects plus 3SD was 6.7 EU and an arbitrary value of <20 EU/mL was set for negative, 20-25 EU/mL indeterminent and >25 EU/mL as positive. A population of 30 patients in the age range at risk for pernicious anemia was tested to validate the established cut-off. All 30 samples were negative.

2. Comparison studies:

a. *Method comparison with predicate device:*
Testing was performed on 47 specimens that included 14 from healthy individuals, 24 from suspected pernicious anemia (PA) patients, and 9 specimens from patients with confirmed PA.

		DPC Intrinsic Factor Antibody **RIA**		
		Positive	Negative	Total
ImmuLisa Anti-Intrinsic Factor Antibody **ELISA**	Positive	16	0	16
	Negative	17	14	31
	Total	33	14	47

Positive percent agreement: 16/33 = 48.5%
Negative percent agreement: 14/14 = 100%
Overall agreement: 30/47 = 63.8%

Twenty of the suspected PA patients had B_{12} that exceeded 2000 pg/mL and all were positive with the RIA assay. The 17 samples positive by RIA and negative by ELISA were from patients with B_{12} levels >2000. To evaluate a possible cause

for discrepant results between the IMMCO and DPC method, IMMCO spiked the normal serum samples negative for intrinsic factor antibodies on both methods with vitamin B_{12}. The spiked specimens were tested for vitamin B_{12} levels and for intrinsic factor antibodies on the RIA and ELISA systems.

#	B_{12} levels unspiked (pg/mL)	B_{12} levels spiked (pg/mL)	RIA unspiked (ratio)	RIA spiked (ratio)	ELISA unspiked (EU/mL)	ELISA spiked (EU/mL)
1	463	>2000	0.92 negative	2.84 positive	17 negative	17 negative
2	374	>2000	0.97 negative	2.11 positive	<13 negative	<13 negative
3	257	>2000	1.04 negative	1.63 positive	<13 negative	<13 negative

This study suggests that the IMMCO ELISA is specific for intrinsic factor antibodies and that a vitamin B_{12} inhibition assay such as the DPC system provide false positive results when free vitamin B_{12} levels in the serum are high. The DPC labeling carries a limitation to this effect.

The 17 discrepant samples in the method comparison study were most likely indicative of patients receiving B_{12} therapy. Literature indicates that B_{12} therapy is known to cause false positive results on RIA test systems.

b. Matrix comparison:

Both assays use serum as the test matrix.

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

A total of 110 samples were tested including 20 PA, 20 healthy controls and 70 disease controls.

Pernicious anemia, other diseases and normal subjects	**N =**	**IF ELISA positive**	**IF ELISA negative**
Pernicious anemia	20	20	0
Rheumatoid arthritis,	10	0	10
Celiac disease,	10	0	10
Hashimoto's thyroiditis,	10	0	10
Graves' disease	10	0	10
H. pylori,	11	4	7
Hepatitis C	19	0	19
Normal	20	0	20
Total	**110**	**24**	**86**

Calculations

		Pernicious Anemia disease status		
		Positive	Negative	Total
ImmuLisa Anti-Intrinsic Factor Antibody test result	Positive	20	4	24
	Negative	0	86	86
	Total	20	90	110

Clinical sensitivity: 20/20 = 100%
Clinical specificity: 86/90 = 95.6%
Overall agreement: 106/110 = 96.4%

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

4. Clinical cut-off**:**
   See assay cut-off.
5. Expected values/Reference range:
   The expected result in the normal population is negative (≥ 20 Units). However, 0.1 – 0.2% of apparently healthy, asymptomatic individuals may test positive for anti-intrinsic factor autoantibodies.

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.