

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE**

A. 510(k) Number:

k033603

B. Analyte:

Type I Intrinsic factor antibody

C. Type of Test:

Competitive immunoenzymatic assay

D. Applicant:

Beckman Coulter

E. Proprietary and Established Names:

Intrinsic Factor Ab Assay, Calibrators, and QC material

F. Regulatory Information:

1. Regulation section:

21 CFR § 862.1810, Vitamin B₁₂ test system
862.1660, Quality control material
862.1150, Calibrator

2. Classification:

Class II, I, II

3. Product Code:

LIG, Radioassay, Intrinsic factor blocking antibody
JJX, Single analyte controls
JIT, Secondary calibrator

4. Panel:

Clinical Chemistry (75)

G. Intended Use:

1. Indication(s) for use:

The Access Intrinsic Factor Ab assay is for the detection of intrinsic factor antibody in human serum and plasma using the Access Immunoassay systems.

Intrinsic factor antibodies are measured as an aid in the diagnosis of pernicious anemia.

2. Special condition for use statement(s):

For professional use only.

Plasma samples used in this assay should be heparinized plasma samples only.

3. Special instrument Requirements:

Access[®] Immunoassay System (comprised of Access, Access 2, Synchron LXi 725, and UniCel DxI 800 analyzers)

H. Device Description:

The Intrinsic Factor Ab Assay consists of 3 wet reagents provided in a ready-to-use test pack for use on Access Immunoassay analyzers. The reagents are as follows:

1. Paramagnetic particles coated with goat anti-mouse IgG, mouse monoclonal anti-intrinsic factor antibodies, buffer, surfactant, and preservative.
2. Porcine intrinsic factor-alkaline phosphatase conjugate complex, buffer, surfactant, and preservative.
3. Buffered protein blocking solution with preservative.

I. Substantial Equivalence Information:

1. Predicate device name(s):
DPC IF bAb
2. Predicate K number(s):
K811927
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Detection of intrinsic factor antibody in human serum and plasma	Detection of intrinsic factor blocking antibody in serum
Antibody measured	Intrinsic Factor	Intrinsic Factor
Format	Competitive	Competitive
Differences		
Item	Device	Predicate
Methodology	Automated enzyme immunoassay	Manual radioimmunoassay
Calibration	Single point stored calibration curve	Single point, no stored calibration
Sample Type	Serum and plasma	Serum
Antibody Type	Monoclonal	None

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

ANSI/ISO/ASQC A3534-1-1993

NCCLS EP7-P - Interference Testing in Clinical Chemistry

NCCLS EP5-A - Evaluation of Precision Performance of Clinical Chemistry Devices

NCCLS H18-A2 - Procedures for the Handling and Processing of Blood Specimens

K. Test Principle:

To measure Intrinsic factor antibody, sample is mixed with the blocking solution and the intrinsic factor-alkaline phosphatase conjugate solution. The mixture is incubated to promote binding of the intrinsic factor conjugate by intrinsic factor antibodies in the sample. The anti-intrinsic factor antibody-coated paramagnetic beads are then added and incubated. The antibodies on the beads compete with the antibodies in the sample for labeled intrinsic factor conjugate. The unbound enzyme conjugate is washed away as the beads are held in a magnetic field. Chemiluminescent enzyme substrate is added and the emitted light is measured. Light production is inversely proportional to the concentration of intrinsic factor antibody in the sample (reported as AU/mL = Antibody Units/mL).

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

To evaluate assay imprecision, five levels of in-house controls were assayed in duplicate for 28 days (according to NCCLS EP5-A guidelines). Results are summarized below.

Sample	Mean (AU/mL)	Within Run SD	Within Run %CV	Between Run SD	Between Run %CV	Total %CV
Level 1	1.06	0.02	1.46	0.05	4.36	4.59
Level 2	1.27	0.02	1.28	0.06	4.58	4.75
Level 3	1.61	0.02	1.34	0.08	4.69	4.88
Level 4	4.63	0.07	1.48	0.23	5.07	5.28
Level 5	14.19	0.21	1.46	0.67	4.71	4.93

Dilution recovery was evaluated using 2 serum samples. Dilutions were made and assayed in replicates of 4. The results (below) show that samples go from positive to negative upon dilution.

Serum Sample 1		
Dilution Factor	Concentration (AU/mL)	Results
Neat	25.67	Positive
1/5	15.71	Positive
1/10	10.90	Positive
1/25	3.45	Positive
1/50	1.72	Positive
1/100	1.47	Equivocal
1/250	1.11	Negative
1/500	1.04	Negative
1/1000	1.02	Negative

Serum Sample 2		
Dilution Factor	Concentration (AU/mL)	Results
Neat	35.29	Positive
1/5	20.66	Positive
1/10	13.24	Positive
1/25	3.76	Positive
1/50	2.14	Positive
1/100	1.6	Positive
1/250	1.2	Equivocal
1/500	1.08	Negative
1/1000	1.03	Negative

b. Linearity/assay reportable range:

This assay reports a ratio that is assigned based on a zero calibrator, and is designated as Antibody Units/mL (AU/mL). The zero calibrator is assigned the value of 1.00 AU/mL. Values less than 0.90 AU/mL should be inspected for contamination.

c. Traceability (controls, calibrators, or method):

The commercially available calibrators are traceable to an internal reference calibrator that is prepared gravimetrically. The Access Intrinsic Factor Calibrator is a single level liquid calibrator containing buffer, human serum albumin, sodium azide, and ProClin300. The calibrator is assigned a value of 1.00 AU/mL in a series of qualification assays. The calibrator value is provided to the user on a card that can be bar-coded into the Access analyzer systems. Calibration is specified every 14 days.

The Access Intrinsic Factor Ab QC material consists of two levels of control material with targeted values of 1.05 and 2.00 AU/mL. The controls consist of intrinsic factor antibody in human serum with mouse proteins, sodium azide and ProClin 300.

Stability studies for the calibrator and control materials are described and are currently ongoing.

d. Detection limit:

Not applicable. This is not a quantitative test.

e. Analytical specificity:

Normal human serum was spiked with the compounds below and compared to the neat sample values to determine potential interference. No significant interference was seen at the levels tested.

Potential Interferent	Concentration Added	Expected (AU/mL)	Observed (AU/mL)	Mean % Recovery
Hemoglobin	500 mg/dL	0.95	1.02	107 %
Bilirubin	20 mg/dL	0.95	0.99	104 %
Lipemia (triolein)	1800 mg/dL	0.95	0.97	102 %
HSA	9 %	1.01	1.00	99 %

Potential interference of naturally occurring vitamin B₁₂ was evaluated by measuring Intrinsic factor antibody in 12 patient samples with vitamin B₁₂ concentrations between 700 and 1600 pg/mL (as measured by the sponsor's vitamin B₁₂ assay) in duplicate and comparing those values to Intrinsic factor antibody concentrations obtained with a commercially available RIA kit. No significant interference was seen up to 1500 pg/mL of vitamin B₁₂.

Potential interference of free vitamin B₁₂ levels was evaluated by spiking various levels of vitamin B₁₂ (as measured by the sponsor's Vitamin B₁₂ assay) into the assay's calibrator (contains no intrinsic factor antibody – AU/mL = 1.00). Results are summarized below:

Sample #	Vitamin B12 (pg/mL)	Access IFAb Result (AU/mL)	Access Result
1	1.5	1.00	Negative
2	104	1.05	Negative
3	163	1.12	Negative
4	189	1.15	Negative
5	201	1.19	Negative
6	255	1.23	Equivocal
7	279	1.27	Equivocal
8	292	1.30	Equivocal
9	322	1.32	Equivocal
10	348	1.40	Equivocal
11	365	1.41	Equivocal
12	444	1.47	Equivocal
13	447	1.54	Positive
14	471	1.58	Positive
15	521	1.59	Positive
16	530	1.71	Positive

Negative samples did not test positive until free vitamin B₁₂ levels were more than 444 pg/mL. This information is in the product labeling along with a caution that this assay should not be used on patients that have received vitamin B₁₂ injection therapy within the previous week.

To assess the effect of other interferences, 87 patient samples were assayed with the device and compared to measurements by the predicate device. The three discrepant samples (in the rheumatoid arthritis patients) were found to be positive in a different commercially available intrinsic factor RIA kit. A statement regarding possible interference with autoimmune disease was added to the labeling. Results are summarized below:

Disease	# of Samples	Access IFAb		Commercial RIA	
		Positive	Negative	Positive	Negative
Rheumatoid arthritis	15	5	10	2	13
Diabetes	4	0	4	0	4
Graves Disease	5	0	5	0	5
Hashimoto's Thyroiditis	5	0	5	0	5
Thyroglobulin Ab	5	0	5	0	5
Low TSH	4	0	4	0	4
HAMA	24	0	24	0	24
Heterophiles	25	0	25	0	25
Totals	87	5	82	2	85
Overall Agreement = 96.6 %					

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

Sixty-seven (67) positive and 60 negative stored samples (as originally determined by the predicate device) were assayed by the device for Intrinsic Factor Antibodies. These samples were also re-assayed with the predicate device. The results are as follows:

		Predicate Device		
		Negative	Indeterminate	Positive
Access	Negative	61	6	2
	Equivocal	0	3	0
	Positive	0	2	53

Negative Agreement: 100 %

95% CI = 94.1 % - 100 %

Positive Agreement: 96.4 %

95% CI = 87.5 % - 99.6 %

Overall Agreement: 92.1 %

95% CI = 86.0 % - 96.2 %

b. Matrix comparison:

To evaluate equivalency of serum and heparinized plasma in the assay, 48 matched serum and heparin plasma samples from apparently healthy adults were tested for each sample type. In addition, 20 samples were spiked with intrinsic factor antibodies to yield a positive result. 100% agreement of positive and negative results was observed.

3. Clinical studies:

- a. *Clinical sensitivity:*
Not applicable.
- b. *Clinical specificity:*
Not applicable.
- c. *Other clinical supportive data (when a and b are not applicable):*
Not applicable.

4. Clinical cut-off:

The sponsor has defined three patient status levels with respect to the detection of Intrinsic factor antibodies. Results <1.20 AU/mL are reported as Negative, results ≥ 1.20 and less than 1.53 AU/mL are reported as Equivocal, and results ≥ 1.53 AU/mL are reported as Positive. The negative cutoff (1.20 AU/mL) was determined from the 99th percentile URL and the positive cutoff (1.53 AU/mL) was determined as the point at which sensitivity and specificity were maximized. This was determined by ROC analysis of 499 samples ranging from 0.93 to 12.25 AU/mL. Sensitivity and specificity are relative to the predicate device.

5. Expected values/Reference range:

The Upper Reference Limit for normals (URL) was determined by measuring the Intrinsic factor antibody in serum samples from 200 apparently healthy individuals. The URL non-parametric 99th percentile was determined to be 1.20 AU/mL.

M. Conclusion:

I recommend that the Access Intrinsic Factor Ab Assay, Calibrators, and QC material assay is substantially equivalent to the legally marketed predicate device.