

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k091468

B. Purpose for Submission:

New device

C. Analyte:

Acetylcholine receptor autoantibodies

D. Type of Test:

Semi-quantitative, Radioimmunoassay

E. Applicant:

IVD Technologies

F. Proprietary and Established Names:

Acetylcholine Receptor AB RIA Kit (AChRAb Assay)

G. Regulatory Information:

1. Regulation section:
21CFR §866.5660, Multiple autoantibodies immunological test system
2. Classification:
Class II
3. Product code:
NST, Autoantibodies, Acetylcholine Receptor, Acetylcholine Blocking and Non-Blocking
4. Panel:
Immunology (82)

H. Intended Use:

1. Intended use(s):
The AchRAb Assay is a radioimmunoassay for the *in vitro diagnostic* semi-quantitative determination of autoantibodies against the acetylcholine receptor in human serum. The AchRAb assay is useful as an aid in the differential diagnosis of Myasthenia Gravis (MG) in conjunction with other clinical and laboratory findings.
2. Indication(s) for use:
Same as intended use
3. Special conditions for use statement(s):
Prescription use only
4. Special instrument requirements:
Gamma counter for I¹²⁵

I. Device Description:

The Acetylcholine Receptor (AchRAb) kit consists of the following:

1. Human Acetylcholine Receptor, labeled with ¹²⁵I α-bungarotoxin, lyophilized
2. ¹²⁵I-AchR Diluent [contains 0.05M Tris buffer with 1.5% surfactant]
3. Standards (6 Levels): 0.00 (normal human serum), 0.2, 0.50, 1.25, 2.50, and 7.5 nmol/L human anti-acetylcholine receptor in human serum; <0.01% NaN₃
4. Positive Control, contains human anti-acetylcholine receptor in human serum, with <0.01% NaN₃

5. Negative Control Cut Off Point, contains human anti-acetylcholine receptor in human serum, with <0.01% NaN₃
6. Goat anti-human IgG, contains <0.01% NaN₃
7. Normal human serum (for diluting test sera), contains <0.01% NaN₃
8. Wash buffer [contains 0.05M Tris buffer with 1.5% surfactant]

J. Substantial Equivalence Information:

1. Predicate device name(s):
IBL ¹²⁵I Acetylcholine Receptor Antibody Kit
2. Predicate 510(k) number(s):
k051144
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use/ Indications for Use	Determination of autoantibodies against human acetylcholine receptor, aid in the differential diagnosis of Myasthenia Gravis	Same
Test principle	Radioimmunoassay (radioreceptor assay)	Same
Test platform	Radiolabeled receptor binds to serum antibodies in liquid phase, antibody-antigen complexes are precipitated and radioactivity measured	Same
Detection instrument	Gamma counter	Same
Calculation	Semi-quantitative determination with standard curve	Same
Quality Control	Positive control and cut-off control	Same

Differences		
Item	Device	Predicate
Sample Types	Serum	Serum and plasma
Negative cutoff	≤ 0.25 nmol/L	≤ 0.25 nmol/L
Equivocal	0.25 – 0.5 nmol/L	0.25 – 0.4 nmol/L
Positive	≥ 0.5 nmol/L	≥ 0.4 nmol/L
Limit of quantitation	0.1 nmol/L	0.01 nmol/L
Assay range	0.1 – 7.5 nmol/L	0.25 – 1.5 nmol/L

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

None referenced.

L. Test Principle:

Patient specimens, standards, and controls are incubated with acetylcholine receptor (AChR) which has been labeled with ^{125}I - α -bungarotoxin. The resulting bound complexes of labeled AChR and autoantibodies are then immunoprecipitated with anti-human IgG. After centrifugation, the supernatant is aspirated and the pellet containing labeled AChR/autoantibody-bound complexes is counted in a gamma counter. Counts are directly proportional to the amount of autoantibodies present.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
 - a. *Precision/Reproducibility:*

Intra-assay

Two different intra-assay precision studies were performed. In the first study, two different technicians ran 16 replicates of three different serum samples. Results are summarized in the following table:

Sample	Tech 1				Tech 2			
	N	Mean (nmol/L)	SD (nmol/L)	CV(%)	N	Mean (nmol/L)	SD (nmol/L)	CV(%)
1	16	0.37	0.033	9.0%	16	0.46	0.028	6.1%
2	16	1.67	0.19	11.3%	16	1.65	0.20	12.2%
3	16	6.45	0.19	2.9%	16	5.91	0.20	3.3%

In the second study, one technician ran 20 replicates of 8 different serum pools. The following table summarizes the results:

Serum Sample	N	Mean (nmol/L)	SD (nmol/L)	CV(%)
Pool 1	20	0.22	0.025	11.3%
Pool 2	20	0.44	0.027	6.2%
Pool 3	20	0.71	0.048	6.8%
Pool 4	20	0.92	0.037	4.1%
Pool 5	20	1.54	0.084	5.5%
Pool 6	20	2.02	0.088	4.4%
Pool 7	20	4.22	0.130	3.1%
Pool 8	20	6.66	0.175	2.6%

Inter-assay

Inter-assay variation was assessed by analyzing 8 serum samples over 15 assay runs. The assays were performed in different labs at the manufacturer's facility with multiple technicians with one kit lot. The mean coefficient of variation was found to be 8.1% (range 4.2 – 10.3%) Results are shown in the table below.

Serum Sample	N	Mean (nmol/L)	SD (nmol/L)	CV(%)
Pool 1	15	0.24	0.024	10.1%
Pool 2	15	0.46	0.047	10.3%
Pool 3	15	0.78	0.058	7.5%
Pool 4	15	1.00	0.092	9.2%
Pool 5	15	1.50	0.14	9.1%
Pool 6	15	2.11	0.21	9.9%
Pool 7	15	4.30	0.20	4.8%
Pool 8	15	6.81	0.29	4.2 %

Lot-to-lot

Lot-to-lot variation was assessed by testing 9 low value serum samples in duplicate on three different kit lots. The mean coefficient of variation was found to be 8.6% (range 3.0-16.6%). Results are summarized in the table below.

Serum Sample	Mean (nmol/L)	CV(%)
A	0.22	16.6%
B	0.17	8.8%
C	0.39	10.5%
D	0.32	3.9%
E	0.58	6.7%
F	0.48	9.2%
G	0.71	5.1%
H	1.30	3.0%
I	1.21	5.5%

b. Linearity/assay reportable range:

Two serum samples with different acetylcholine receptor levels were diluted with the zero calibrator, in ratios of 1:1.2, 1:1.4, 1:1.8, 1:2, 1:3, 1:4, and 1:8. The graph of measured vs. expected results had slope = 0.989, intercept = 0.032, and $r^2 = 0.993$. The results are summarized in the table below. The assay is linear up to 7.5 nmol/L, the level of the highest calibrator.

Serum Sample—Pool 8			Serum Sample—Pool 2		
Dilution	Measured (nmol/L)	Recovery (%)	Dilution	Measured (nmol/L)	Recovery (%)
Undiluted	>8.5	--	Undiluted	0.72	106
	>8.5	--		0.64	94
1:1.2	9.14	103	1:1.2	0.54	95
	8.12	92		0.49	86
1:1.4	7.63	101	1:1.4	0.54	111
	7.56	100		0.40	82
1:1.8	6.61	112	1:1.8	0.39	103
	5.99	101		0.35	93
1:2	6.40	120	1:2	0.28	82
	5.88	111		0.30	88
1:3	3.95	112	1:3	0.24	106
	3.47	98		0.21	96
1:4	2.80	105	1:4	0.14	82
	2.76	104		0.15	88
1:8	1.36	102	1:8	0.10	118
	1.17	88		0.10	118

Recovery: Increasing amounts of acetylcholine receptor antibodies were added to three different serum samples with various initial acetylcholine receptor antibody levels, resulting in 9 different pools overall. Each sample was assayed in quadruplicate. The mean recovery ranged 94% to 105%.

Spiked Sample	Expected (nmol/L)	Recovered (nmol/L)	Recovery (%)
A	0.86	0.81	94%
B	1.13	1.09	97%
C	1.29	1.21	94%
D	1.55	1.49	96%
E	2.08	2.13	102%
F	2.42	2.36	97%
G	3.69	3.89	105%
H	4.15	4.27	103%
I	4.64	4.85	105%

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Traceability: No reference material is available.

Stability: Real-time stability testing of two lots of the AChRAb tracer (human acetylcholine receptor labeled with ¹²⁵I α-bungarotoxin) demonstrated that the unopened reagent is stable for three months at 2-8°C. Other kit reagents are stable for at least 12 months at 2-8°C. The reconstituted tracer is stable for at

least 30 days at 2-8°C.

Complete unopened kits were placed at different temperatures to evaluate the effect of extreme temperatures. The kits are stable for up to 30 days at 25°C, at least 9 days at -10°C and 37°C, and up to 6 days at 41°C.

d. Detection limit:

The Limit of Blank was determined to be 0.058 nmol/L. Functional sensitivity (Limit of Quantitation) was determined to be 0.1 nmol/L.

e. Analytical specificity:

Cross-reactivity with autoantibodies potentially interfering with the AchRAB Assay was assessed. Eight patient serum samples containing autoantibodies (anti-Sm/RNP, -Ro (SS-A), -La (SS-B), -dsDNA, -RF, and ANA antibodies) were tested in the assay. No cross-reactivities were found to AchRAB. In addition, hemoglobin (1.9 – 400 mg/dL), bilirubin (1.25 – 50 mg/dL), and triglycerides (25-3000 mg/dL) were evaluated for interference. All test results are available in the submission. Results showed no interference at the concentrations tested.

f. Assay cut-off:

Serum samples from 72 apparently healthy male and female adult blood donors were assayed. The range of values was between 0.0 and 0.22 nmol/L with a mean of 0.082 nmol/L and a standard deviation of 0.061 nmol/L. The cutoff was set at 0.25 nmol/L (approximately the mean + 3SD of the normal range). Antibody titers greater than 0.5 nmol/L are indicative of Myasthenia Gravis (positive). Values between 0.25 and 0.5 nmol/L are considered equivocal.

2. Comparison studies:

a. Method comparison with predicate device:

131 patient samples were tested with the AchRAB test and with the predicate assay. Equivocal results are not counted as positive or negative for the purpose of determining % Agreement.

		Predicate Assay (IBL ARAb)			
		+	Equivocal	-	Total
IVD AchRAB Assay	+	96	1	0	97
	Equivocal	0	0	2	2
	-	1	3	28	32
	Total	97	4	30	131

Treating equivocal results as positive:

Positive agreement (97/101) = 96.0% (95% CI: 90.2 - 98.4)

Negative agreement (28/30) = 93.3% (95% CI: 78.7% to 98.2%)

Overall agreement (125/131) = 95.4% (95% CI: 90.4% to 97.9%)

Treating equivocal results as negative:

Positive agreement (96/97) = 99.0% (95% CI: 94.4% to 99.8%)

Negative agreement (31/34) = 97.1% (95% CI: 85.1% to 99.5%)

Overall agreement (127/131) = 98.5% (95% CI: 94.6% to 99.6%)

b. *Matrix comparison:*

Not applicable.

3. Clinical studies:

a. *Clinical Sensitivity and Specificity:*

40 samples with known clinical diagnosis of Myasthenia Gravis (MG) were tested using the AchRAb kit and the predicate kit. Both the AchRAb kit and the predicate kit correctly identified MG in all 40 samples. In addition, 16 samples from patients diagnosed with other neurological diseases were tested using the AchRAb kit and the predicate kit. All were identified as negative for MG.

		Myasthenia Gravis		
		+	-	Total
IVD AchRAb Assay	+	40	0	40
	Equivocal	0	0	0
	-	0	16	16
	Total	40	16	56

Sensitivity (40/40) = 100.0% (95% CI: 91.2% to 100%)

Specificity (16/16) = 100.0% 95% CI: 80.6% to 100%)

Overall agreement (56/56) = 100.0% (95% CI: 93.6 to 100%)

c. 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 value in normal population is < 0.25 nmol/L.

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.