Evaluation of a Radioimmunoprecipitation Assay for the Detection of Ganglionic Acetylcholine Receptor Antibodies

Thomas R Haven[1], Bucky K Lozier[1], Anne E Tebo[1,2], Lisa K Peterson[1,2]

[1]ARUP Institute for Clinical and Experimental Pathology, [2]Department of Pathology, University of Utah School of Medicine, Salt Lake City, Utah.

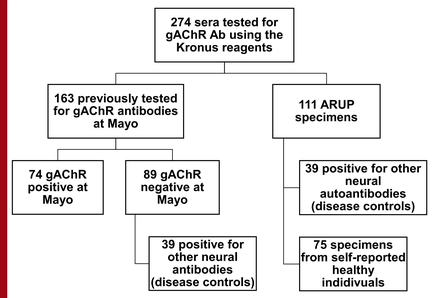
Objective

To evaluate the performance characteristics of a radioimmunoassay (RIA) to detect Ganglionic Acetylcholine Receptor (gAChR) autoantibodies.

Background

Ganglionic acetylcholine receptor (gAChR) antibodies have been detected in a variety of clinical syndromes including autoimmune autonomic ganglionopathy (AAG), stiff person syndrome as well as certain autoimmune rheumatic diseases such as systemic sclerosis patients with gastrointestinal symptoms. These autoantibodies also coexist with striated muscle, neuromuscular acetylcholine receptor and VGKC antibodies seen in a number of autoimmune neurologic neurologic disorders. gAChR antibodies are detected in approximately 2% of patient sera evaluated for paraneoplastic antibodies. Thus, there is a need for testing to be more widely available.

Methods

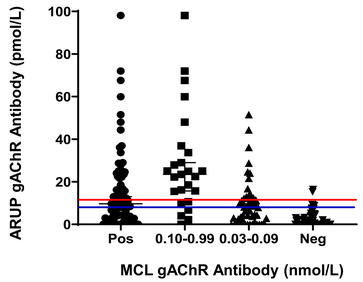


The performance of the gAChR antibody assay was evaluated based on ranges previously established during validation testing (<8.5 pmol/L=negative, 8.5–11.6 pmol/L=indeterminate, >11.6 pmol/L= positive). The comparison was performed using results previously obtained for 163 serum samples previously tested at MCL based on a tiered positive predictive value (PPV) of the predicate assay. Assay specificity was assessed using controls.

Results

Low positive gAChR antibodies demonstrate variable interlaboratory concordance and are not associated with AAG or related diseases

MCL Result Range	PPV	#	% Positive
0.10 - 0.99 nmol/L	50%	25	76%
0.03 - 0.09 nmol/L	46%	49	27%
<0.02 nmol/L	--	89	2%



Comparison of ARUP anti-gAChR results with those of the MCL predicate assay using the reference intervals established during the validation phase [0.0-8.4 pmol/ L= negative (blue line), 8.5-11.6 pmol/L = indeterminate, >11.6 pmol/L= positive (red line)] and those provided on the reports for the predicate assay. MCL result categories were stratified by PPV.

Acceptable results were obtained for all performance characteristics. However, the potential for bilirubin to interfere with the results could not be determined due to interference caused by its diluent.

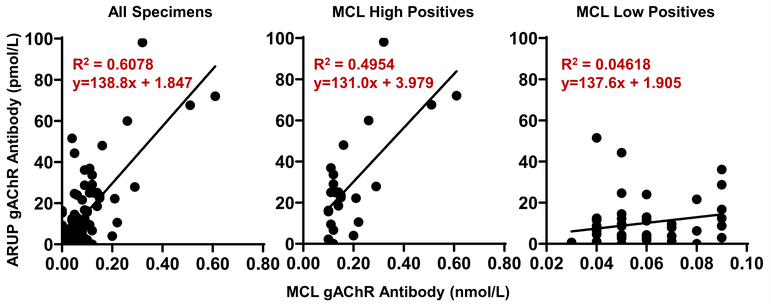
		Negative	Low Positive	High Positive
		Mean CV (Range)	Mean CV (Range)	Mean CV (Range)
Precision	Overall	N/A (N/A)*	14.2% (2.8-28.1%)	7.2% (4.0-13.2%)
	Within Run	N/A (N/A)	16.4% (5.7-28.1%)	4.1% (4.0-4.1%)
	Between Run	134.6% (124.9-144.3%)	10.8% (2.8-18.8%)	10.3% (7.4-13.2%)
		% Difference (Range)	% Difference (Range)	% Difference (Range)
Interference	Hemoglobin	N/A (N/A)	10% (6.6-13.4%)	10.8% (9.0-12.5%)
	Triglycerides	N/A (N/A)	16.2% (14.5-17.9%)	11.2% (4.7-17.6%)
	Bilirubin	Unable to interpret	Unable to interpret	Unable to interpret
Stability	48 hrs RT	N/A (N/A)	16.9% (5.6%-28.1%)	4.4% (0.5-8.2%)
	14 days 2-8 °C	N/A (N/A)	3.9% (2.7-5.0%)	26.5% (17.3-35.7%)*
	30 days -20 °C	N/A (N/A)	5.0% (1.9%-8.0%)	121% (5.9-18.2%)
	3 Freeze/Thaw	N/A (N/A)	10.9% (10.0-11.8%)	14.0% (12.9-15.0%)

*Due to low result values, elevated CVs were observed. However, results were acceptable as qualitative interpretation did not change. N/A = not applicable, these CVs were unable to be calculated due to results of 0.0. Precision was evaluated by testing 2 negative, 2 low positive, and 2 high positive 3 times on one run (within run) and once on two additional runs. Data shown in the table above are the mean variation and range of 2 specimens at each result level. The first result from each of the 3 runs was used to evaluate between run precision. Hemoglobin concentrations up to 1520 mg/dL, triglycerides up to 4474 mg/dL, and free bilirubin up to 13.75 mg/dL had no significant influence on results.

Discrepancies between laboratories involved low positive gAChR results were associated with other immune conditions, primarily autoimmunity, not AAG or related diseases.

Characteristics	
Number, N	31
Age, mean ± SD years	52.9 ± 15.3
Female, %	58.1
Race/Ethnicity (N=31)	
White,	93.50%
Hispanic/Latino	6.50%
Clinical diagnosis (N=31)	
*Autoimmune neurologic, n (%)	22 (71%)
Non-neurologic autoimmune, n (%)	12 (39%)
Autonomic involvement, n (%)	28 (90%)
**Cancer, n (%)	9 (32%)
Neurologic genetic disease, n (%)	1 (3%)
gAChR Antibody Results (N=31)	
Positive in both labs, n (%)	21 (68%)
Positive in MCL, n (%)	29 (94%)
Positive in ARUP, n (%)	21 (68%)
Positive MCL and equivocal ARUP, n (%)	2 (6%)
Positive MCL and negative ARUP, n (%)	6 (19%)
Positive/equivocal ARUP and negative MCL, n (%)	2 (6%)

Quantitative comparison also demonstrates poor correlation for low positive gAChR results



Quantitative comparison of ARUP anti-gAChR results with those of the MCL predicate assay Comparisons were made using all of the specimens tested at both laboratories, as well as MCL positive result categories stratified by PPV [high positive and low positive).

Discussion and Conclusions

- **The gACHR RIA shows good overall agreement with the predicate assay although challenges with low positive results were observed.**
- **In agreement with the literature, it was not unusual for gACHR antibody to co-exist with other antibodies. Descending order in our cohort were GAD65, P/Q-VGCC, VGKC, Striational, muscle ACHRBIN N-VGCC and GABA-B. Some sera possessed multiple antibodies.**
- **Based on available clinical information, discrepancies between the two laboratories involved low positive gAChR results which were associated with other immune conditions, primarily autoimmunity, not autoimmune autonomic ganglionopathy (AAG).**
- **The gAChR RIA demonstrated good stability and reproducibility and the presence of hemoglobin and triglycerides did not have a significant impact on results.**
- **The overall inter-laboratory correlation was comparable (0.78); however, challenges were observed for low positive results.**
- **Collaborative efforts aimed at assessing the clinical spectrum associated with these antibodies and harmonizing testing are required.**

Acknowledgements

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