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# Detection of autoantibodies against the acetylcholine receptor, evaluation of commercially available methodologies: Cell-Based Assay, Radioimmunoprecipitation and ELISA

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## Background

Myasthenia Gravis (MG) is an autoimmune disease resulting from the action of pathogenic autoantibodies (AAbs) directed against nicotinic acetylcholine receptors (AChR), which interfere with communication between the neurotransmitter acetylcholine and its receptor on the muscle fiber. The detection of anti-AChR using RadioImmunoPrecipitation Assay (RIPA) has 100% specificity for the diagnosis of MG, however RIPA has high execution and interpretation complexity and requires radioactive materials, which restrict their use to specialized laboratories.

## Objective

Our goal was to compare the performance of different commercial assays to detect anti-AChR AAbs: the gold standard RIPA, a recently marketed cell-based assay (CBA), and two solid-phase ELISA kits.36

## Results

Table 1. Reactivity to AChR and HEp-2 cells.

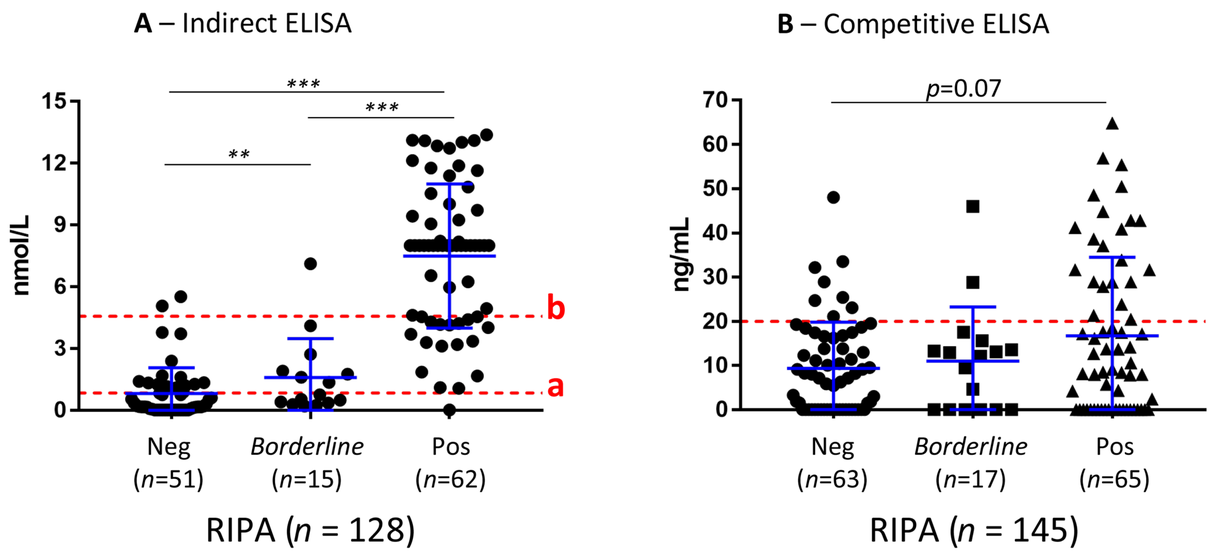
| RIPA (n=145) (Mayo Clinic, USA) | | **Negative** (n=63) <0.02 nmol/L | ***Borderline*** (n=17) 0.22 (±0.2) nmol/L | **Positive** (n=65) 20.1 (±20.3) nmol/L | *p* value | Cohen's Kappa (95% CI) |
|---|---|---|---|---|---|---|
| Indirect ELISA (n=128) (Euroimmun, EA 1435-9601 G) | | (n=51) | (n=15) | (n=62) | | |
| Cutoff (Youden index J = 0.8409) | - | 68.6% (n=35) | 53.3% (n=8) | 1.6% (n=1) | <0.001 | 0.688 (0.557-0.819) |
| | + | 31.4% (n=16) | 46.7% (n=7) | 98.4% (n=61) | | |
| Cutoff (average + 3SD of RIPA-Neg = 4.568) | - | 96.1% (n=49) | 93.3% (n=14) | 29.1% (n=18) | <0.001 | 0.652 (0.519-0.785) |
| | + | 3.9% (n=2) | 6.7% (n=1) | 70.9% (n=44) | | |
| Competitive ELISA (n=145) (Cutoff = 20 ng/mL) (MyBioSource, MBS729942) | - | 87.3% (n=55) | 88.2% (n=15) | 66.2% (n=43) | 0.008 | 0.210 (0.068-0.351) |
| | + | 12.7% (n=8) | 11.8% (n=2) | 33.8% (n=22) | | |
| CBA (n=145) Myasthenia gravis Mosaic 2 (Euroimmun, FA 1435-1010-2) | AChR-ε | 0% (n=0) | 29.4% (n=5) | 96.9% (n=63) | <0.001 | 0.969 (0.928-1.000) |
| | AChR-γ | 0% (n=0) | 23.5% (n=4) | 90.8% (n=59) | | 0.909 (0.839-0.978) |
| | CBA-ε/γ-Neg | 100% (n=63) | 70.6% (n=12) | 3.1% (n=2) | | - |
| HEp-2 IFA (Euroimmun, FA 1520-2010) | Neg, 48.9% (n=71) | 47.6% (n=30) | 41.2% (n=7) | 52.3% (n=34) | 0.687 | - |
| | Pos, 51.1% (n=74) | 52.4% (n=33) | 58.8% (n=10) | 47.7% (n=31) | | |
| Titer | | 1/467 (±483) | 1/271 (±162) | 1/415 (±339) | 0.489 | - |
| Patterns (ICAP) | Nuclear speckled (AC-2, AC-4, AC-5) | 23 (59%) | 8 (62%) | 22 (63%) | 0.948 | - |
| | Nucleolar (AC-8 to AC-10) | 5 (13%) | 1 (8%) | 6 (17%) | | |
| | Cytoplasmic (AC-15 to AC-23) | 7 (18%) | 2 (15%) | 4 (11%) | | |
| | Others (AC-3, AC-7, and AC-25 to AC-28) | 4 (10%) | 2 (15%) | 3 (9%) | | |

## Discussion

- Among the two ELISAs, better results were observed with the indirect ELISA with a cutoff that promotes higher specificity (in this case, 96.0% specificity), but at the expense of low sensitivity (~70% of the RIPA-pos samples);
- We observed a good performance of the fixed CBA for detection of anti-AChR when compared to RIPA, with an almost perfect agreement (Kappa=0.969);
- In addition, there was a good correlation between anti-AChR levels by RIPA and Euroimmun's indirect ELISA results (Spearman r=0.845; p=<0.001).

Ethics committee approval, Plataforma Brasil CAAE: 57480622.3.0000.5474.

**Figure 1. Anti-nAChR reactivity by ELISA.**

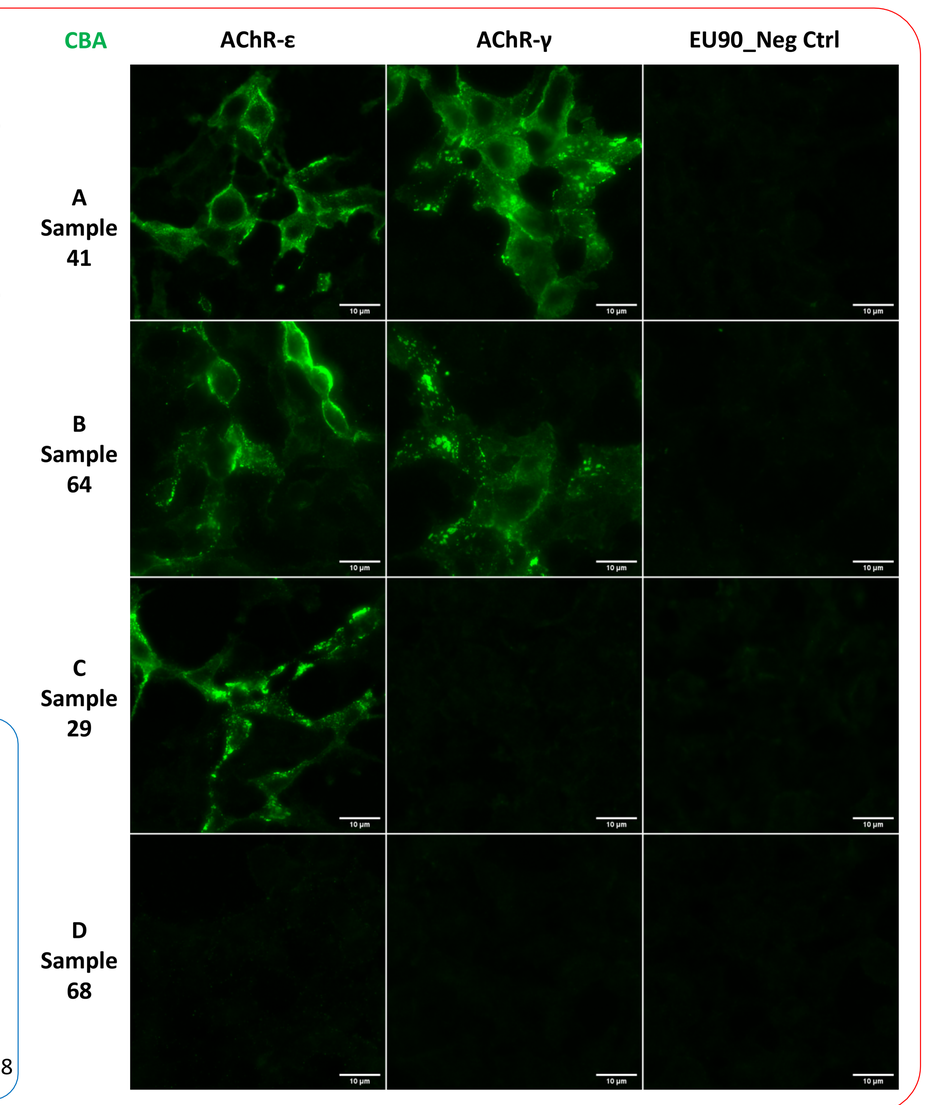



(A) Line "a" indicates the Youden index J cutoff = 0.8409, that promotes sensitivity. Line "b" indicates a cutoff that promotes specificity = 4.568 (average + 3SD of RIPA-Neg) **p≤0.01; ***p≤0.001.

(B) Competitive ELISA. The cutoff of 20ng/mL was based on the average + 1SD of RIPA-Neg group.

**Figure 2. Anti-nAChR reactivity by the fixed Cell-Based Assay (CBA).**

Indirect Immunofluorescence analysis with EU90 cells expressing AChR-ε or AChR-γ.
(A and B) Examples of samples with reactivity for ε and γ.
(C) Example of a sample with reactivity only for ε.
(D) Example of a sample without any reactivity.

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## Conclusion

- The fixed CBA presented better performance than the two ELISAs and showed an almost perfect agreement and 100% specificity compared to the gold standard RIPA test.
- ELISA could be an option to estimate anti-AChR AAb levels after confirming positivity by the CBA.
- This kit was recently launched commercially and is currently in exclusive use for research purposes, but it has promising potential as an alternative to RIPA in the clinical laboratory, especially due to its radiation-free nature.

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