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# Antibody testing for autoimmune neurological disorders: Which tests to order and why?

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Autoimmune neurological disorders are sub-acute and rapidly evolving heterogeneous conditions that can present with a wide spectrum of neurological symptoms [1]. These disorders occur as a result of an abnormal immune response targeting specific antigens in the central and peripheral nervous systems, which are diagnosed by the detection of autoantibodies (AAbs) in the serum or cerebrospinal fluid (CSF). These neural AAbs are classified according to the cellular location of their target antigens [1]; the first AAbs group targets cell-surface antigens including ion channels, water channels and neurotransmitter receptors. Some of the examples of the neuroimmunological disorders associated with this group of AAbs are Myasthenia Gravis (MG), Autoimmune Encephalitis (AE), Neuromyelitis Optica Spectrum Disorder (NMOSD), Chronic Inflammatory Demyelinating Polyradiculoneuropathy (CIDP). The second group of AAbs targets intracellular antigens and are frequently associated with cancer, known as classic paraneoplastic neurological syndrome (PNS). The clinical diagnosis is often difficult due to the overlap of symptoms and AAbs profiles among different phenotypes. On the other hand, the laboratory testing has become increasingly complex primarily due to continuous discovery of novel AAbs and the availability of high sensitivity clinical assays. Nevertheless, in most cases, an early detection and prompt therapy can improve patient outcome. In addition, if correctly diagnosed, the PNS associated AAbs are extremely useful biomarkers to search for the underlying cancer.

Regarding AAbs testing, most clinical laboratories use commercial immunofluorescence (IFA), ELISA (for cell-surface antigens), western blot or line immunoblots (for intracellular antigens); however, these methodologies when used alone lack the required sensitivity and specificity [1, 2, 3, 4, 5]. In contrast, the radioimmunoprecipitation assays (RIPA), and tissue-based immunohistochemistry/immunofluorescence (IHC/IFA) assays (TBAs) offer excellent specificity and sensitivity; however, these techniques are only available in a few highly specialized neuroimmunology reference laboratories [1, 2]. More recently, the novel live cell-based assays (CBA) are getting increasingly popular in neuroimmunology testing labs to diagnose cell-surface AAbs, with better sensitivity and specificity than the RIPA assay [1, 4, 5]. In a recent study from our laboratory, we re-assayed 17 seronegative, clinical MG positive cases (acetylcholine receptor, AChR AAbs negative by RIPA) and found 23% (4/17) positive for AChR AAbs with a novel live CBA4. . In the second study from our laboratory, approximately 16% (7/44) of children with seronegative MG were tested positive for AChR AAbs by a live CBA3. All 7 children with positive results were clinically confirmed for acquired MG3. To assure the highest specificity in AAbs testing, we recommend using CBA and an orthogonal reflex-testing algorithm, and if clinically needed, a confirmation test can be performed by an independent method. [1, 2, 5, 6]

We and others have also established CBA superiority for the detection of nodal and paranodal AAbs testing in CIDP patients, particularly in those who are resistant to standard therapy or have atypical phenotypes[6].

In contrast, the intracellular AAbs are frequently tested by commercial line immunoblots, however, the number of false positives is surprisingly high with these line blots [1, 2]. Here our laboratory recommends that each suspected PNS sample should be first screened on snap-frozen rat brain IHC/IFA, TBA assay and then the results should be confirmed on specific

CBA and/or immunoblot assays [1]. Unfortunately, snap-frozen rat brain HC/IFA TBA testing is available only in a few highly specialized neuroimmunology laboratories1.

In addition, it is important to know when it is right to send serum and when to include CSF for testing, as sensitivity and specificity for serum and CSF testing vary among different AAbs. Some AAbs are more readily detected in serum than CSF (for example, AChR Abs, aquaporin 4 (AQP4)-in NMO and LGI1-in AE). In contrast, for detecting intrathecally produced neural cell-surface AAbs (for example, NMDA-in AE), the CSF testing is crucial as it is more sensitive and specific.

In conclusion, although a wide range of clinical assays are available, it is important to realize that not all AAbs are same in terms of their clinical utility and no single test is accurate enough to diagnose all autoimmune neurological disorders. Thus, a phenotype-based evaluation and orthogonal testing based on AAbs profile is a preferred laboratory diagnostic strategy. Any unexpected AAbs positive results with discordant clinical phenotypes from a clinical laboratory should be reassessed using a comprehensive testing scheme at a specialty laboratory. Similarly, all seronegative but clinically suspected patient samples should be re-evaluated and each test results should always be interpreted within their clinical context.

## REFERENCES


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