Simplifying the complexity of autoimmune neurological diseases

Testing methodologies that help physicians **accelerate the path to diagnosis**

40 years of rapid discovery, research, and innovation

Antibody testing for autoimmune neurological disorders is a rapidly growing field, with the term "neuroimmunology" first used in PubMed in 1982.[1] The number of antibodies being discovered that are biomarkers for these diseases continues to grow, with the discovery of >20 antibodies that play a role in autoantibody-mediated neurologic diseases since 2004.[2]

Multiple testing methodologies exist, but testing for these diseases has become increasingly complex due to the growing number of antibodies that are biomarkers and the nature of the diseases themselves. A patient may often manifest a variety of symptoms that could be caused by 1 or more autoantibodies. The complexity of the neurological system and the absence of a specific clinical presentation make it difficult to know where to look first. In most cases, early detection and prompt therapy can improve patient outcomes[3]—which makes utilizing the right test, at the right time, for the right patient crucial to giving patients hope and helping physicians find the right pathway forward.

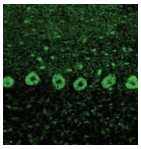
The starting point: Tissue immunofluorescence assay

Testing for autoantibodies using tissue immunofluorescence assay (IFA) is an effective first approach for laboratory evaluation of a patient suspected of having a neurological autoimmune disease. Tissue IFA detects the antibody binding to a tissue composite, which allows us to see certain patterns indicating what the antibody might be. Primate or rat tissue may be used with this method.

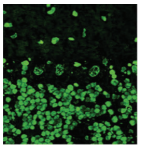
At Quest Diagnostics®, we use primate tissue (cerebellum, nervus suralis) as neuronal tissues, as they are most similar to human tissue and help reduce background noise and improve specificity. We then compare what we see with other primate tissues (intestine, kidney, testis, pancreas) because some of the neuronal-specific antibodies target these other tissues. This process helps to differentiate antibodies based on tissue staining, aids in narrowing our search to more specific analytes, and enables a more in-depth examination of different patterns. Additionally, we include a HEp-2 cell—which is the traditional substrate for classical anti-nuclear antibody IFA—as a control. This helps us determine neuronal specificity.

Confirmatory testing for IFA: Western line blot

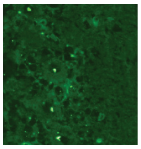
Western line blot is an intracellular testing methodology that confirms tissue IFA patterns by placing purified target proteins on a membrane that's optimized for that particular protein. We use densitometry to quantify the amount of antibody binding to confirm tissue IFA patterns and facilitate a diagnosis. This testing methodology is only run as a reflex based on the initial IFA result, ensuring we are performing only clinically relevant tests to avoid unnecessary costs.



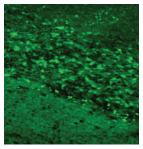
Purkinje cells:
Yo
Tr (DNER)
ITPR1
CARPVIII



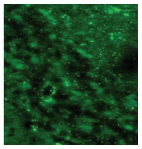
Neuronal nuclear:
Hu
Ri
Zic4
Anna-3



Neuronal nuclear:
Ma1
Ma2/Ta



Intracellular synaptic:
Amphiphysin
GAD



Intracellular:
CV2

Coming into focus: Cell-based assay and radioimmunoassay

When performing a cell-based assay (CBA), we recombinantly express the protein on a cell to determine whether antibodies that bind to those cells are expressing the protein of interest. The detection of central nervous system (CNS) autoantibodies is generally better achieved with CBAs—65% of autoantibodies are missed when testing by IFA alone.[4] At Quest, a CBA is always run as part of the initial panel, increasing the likelihood of identifying membrane-embedded protein targets.

There are 2 types of CBA testing—live and fixed-based. Fixed-based CBA offers some distinct advantages over live CBA:

- More reproducible
- More cost efficient
- Fewer false positives
- Better specificity for some antibodies such as MOG[5]

Radioimmunoassay (RIA) is a sensitive assay technique that measures concentrations of antigens using antibodies directed against these antigens. Once a specific antibody has been identified, we can retest using RIA to monitor that patient's progress and response to treatment. If a patient relapses or presents with a flare-up, RIA testing is extremely useful to determine current patient antibody levels and compare those against past results.

In focus: Integrated test strategies that streamline the path to a diagnosis

Leveraging multiple testing methodologies that complement one another and assess clinically relevant autoantibodies may

Identify idiopathic neurological disease in the absence of a tumor

Identify a malignancy early to optimize treatment and help improve outcomes

Inform targeted immunosuppressive therapy for immunological disease

A patient-centric approach can help accelerate diagnosis

When a patient presents with a range of neurological symptoms, it can be difficult to determine their cause. Clinical presentations do not always correlate with classic descriptions of autoantibody-associated diseases. In a study of 16,700 samples tested for neurological autoantibodies, approximately 50% tested positive for autoantibodies other than those included in the initial testing order.[6] By screening for multiple autoantibodies, the detection rate for diagnostically relevant autoantibodies increased by 87% compared to testing of requested analytes.[6]

Know where to look first with advanced neuroimmunology testing

While the diseases themselves are complex, testing for them shouldn't be. Neuroimmunology testing that leverages advanced technologies and focuses on specific antibodies known to be associated frequently with neurological disorders is crucial to help lend clarity to the complexity of these diseases.

Comprehensive neuroimmunology testing from Quest Diagnostics

Demyelinating disorders, including neuromyelitis optica spectrum disorder (NMOSD), myelin oligodendrocyte glycoprotein (MOG) antibody disease, and multiple sclerosis (MS)

NMOSD is an immune-mediated, chronic, and often relapsing inflammatory disease that can sometimes be mistaken for MS because many of the symptoms between the 2 diseases overlap. Early diagnosis is critical because treatments for MS may be ineffective or harmful for a patient with NMOSD.[7]

MOG is a demyelinating disorder characterized by attacks of optic neuritis, myelitis, brain or brainstem inflammation, or combinations of the above.[5]

- The **NMO Spectrum Evaluation** reflexes to MOG if AQP4 is negative, as research indicates 15% to 35% of patients with NMO phenotype who test negative for AQP4 test positive for MOG antibodies[8]—ensuring we are performing only clinically relevant tests to avoid unnecessary costs

Myasthenia gravis (MG)

MG is an autoimmune disorder characterized by weakness of skeletal muscle that ranges from mild to severe for multiple voluntary muscle groups and most commonly involves AChR or MuSK antibodies.

We offer the largest range of antibody testing for MG, testing for 5 known antibodies (AChR, MuSK, LRP4, RyR, and titin) to help guide treatment options.

A way forward: LRP4 testing

LRP4 antibodies are believed to be the third-leading cause of MG.[9] LRP4 antibodies were found in 13% of patients testing negative for other MG antibodies.[10]

- Most LRP4-positive patients improve after standard MG therapy[10]
- LRP4 is most prevalent in ocular MG[11]

Paraneoplastic neurological syndromes (PNS), including autoimmune encephalitis and epilepsy

PNS are a group of uncommon disorders that develop in some people who have cancer. In 60% of patients with PNS, the symptoms occur before the cancer is diagnosed.[12] For autoimmune encephalitis and epilepsy, an early diagnosis is crucial and can help a patient regain motor, executive, and cognitive functions.

- The **Paraneoplastic Antibody Expanded Evaluation** has the ability to identify 25 prevalent antibodies for increased sensitivity, including Ma2/Ta and Zic4
- A CBA is always run as part of the initial panel, increasing the likelihood of identifying membrane-embedded protein targets NMDA (NR1), LGI1, CASPR2, AMPAR, and GABAR
- The **Autoimmune Encephalitis Evaluation** panels from Quest are built on 25 antibodies commonly found in autoimmune encephalitis
- A CBA panel for neuronal cell-surface antigens is always performed and includes NMDA antibodies that are consistent with limbic encephalitis

Peripheral neuropathy

Peripheral neuropathies are diseases or conditions that affect the peripheral nerves, causing numbness, weakness, and burning, tingling, cramping, and/or sharp pain. They may be caused by gene mutations, toxin or drug exposure, physical trauma, or inflammation.

- Symptoms include weakness, numbness, and pain, usually in the hands and feet[13]
- **SensoriMotor Neuropathy Profile with Recombx®-Complete** tests for these top markers related to peripheral neuropathology: MAG, SGPG, GM1, GD1a, GD1b, asialo-GM1, sulfatide, Hu, and IgM GALOP antibodies

Quest Diagnostics®

A case study: What is Ma2/Ta?

While the role of Ma2/Ta in encephalitis is now widely recognized, Quest was one of the first labs to recognize Ma2/Ta and its role in neuroimmunological diseases. Back in 2013, we reviewed a sample in the lab that showed a significantly elevated Ma2/Ta—we had validated the test, but had not yet commercialized it. Our physician reached out to the ordering neurologist and advised him to consider Ma2/Ta. His response was, **"What is Ma2/Ta?"**

While the antibody had been reported in men with testicular cancers, its role in neuroimmunology was still emerging. This patient was a 10-year-old girl, comatose in the ICU. Physicians suspected she had aseptic encephalitis and were working to confirm the diagnosis. By identifying the elevated presence of Ma2/Ta, we were able to help the neurologist confirm the diagnosis and order appropriate immunosuppression therapy, which was administered successfully. During a follow-up with the neurologist 4 years later, we learned the patient had recovered and was now a happy teenager who was doing reasonably well considering her ordeal and diagnosis.

Ma2/Ta case study

10-year-old female patient in ICU, comatose, with physicians needing to confirm a preliminary diagnosis of aseptic encephalitis

Detected **the presence of elevated** Ma2/Ta antibodies

Administered appropriate **immunosuppression therapy**

Patient shows recovery at 4-year follow-up

Unrivaled expertise that helps deliver better outcomes

Our medical and scientific experts—including 700+ MDs, PhDs, and genomic science specialists—have decades of experience interpreting results to help you make the differential diagnoses critical for appropriate treatment. Our team is relentless in our pursuit of answers that inspire life-saving action for patients with neuroimmunological diseases. When we see an interesting or unusual pattern, we investigate further and convene a team of experts to determine the next step. All tissue IFA report-outs undergo a second review by a medical director. We also have a rigorous, 6-month training program for new members of our pathology team, where they are able to review, analyze, and recognize patterns in close collaboration with existing team members and our medical director.

"We recognize behind every sample is a life. For many patients, the road to a diagnosis is not linear and is quite challenging. Untangling the mysteries in neuroimmunology to help a patient overcome the obstacles in their diagnostic journey—so they get the right diagnosis and the right treatment—is what drives us. Our team works every day to deliver actionable insights to give a patient new hope for life-saving answers and treatment."

—Karthik Kuppusamy, PhD, Senior Vice President, Clinical Solutions

Helping your patients get the tests they need—and the answers they deserve

We are committed to helping your health system minimize clinical care variation and support better care decisions. When advanced scientific testing solutions are complemented by industry-leading medical expertise and are easily accessible via LIS/EHR integration, insurance access, and fast results, the path to a diagnosis is illuminated. Testing is performed only at Quest facilities, using gold standard methodologies to ensure test quality, accuracy, rapid turnaround time, and top performance. With turnaround times averaging 3-14 days, you can count on us for fast, accurate results that can help drive early detection and quick treatment—making a difference in patient outcomes and giving patients answers they deserve.

Learn more about why Quest is the lab of choice for neuroimmunology and other advanced diagnostics for so many health systems. Visit **QuestDiagnostics.com/Neuroimmunology**

References

1. Nutma E, Willison H, Martino G, Amor S. Neuroimmunology—the past, present and future. *Clin Exp Immunol.* 2019;197(3): 278-293. doi:10.1111/cei.13279 **2.** Woodhall M. Utility of live cell-based assays for autoimmune neurology diagnostics. *J Appl Lab Med.* 2022;7(1): 391-393. doi:10.1093/jalm/jfab133 **3.** Vincent A, Bien CG, Irani SR, Waters P. Autoantibodies associated with diseases of the CNS: new developments and future challenges. *Lancet Neurol.* 2011;10(8):759-772. doi:10.1016/S1474-4422(11)70096-5 **4.** Abelhosn RW, Montana L, Rivera JG, et al. Tissue immunofluorescence confirmation of CNS autoantibodies identified by immunoblot or cell-based assay. *J Neurosci Neuropsych.* 2021;103:4. Online ahead of print, December 15, 2020. doi:10.21203/rs.3.rs-126755/v1 **5.** Sechi E, Buciuc M, Pittock SJ, et al. Positive predictive value of myelin oligodendrocyte glycoprotein autoantibody testing. *JAMA Neurol.* 2021;78(6):741-746. doi:10.1001/jamaneurol.2021.0912 **6.** Stocker W, Probst C, Teegen B, et al. Multiparameter autoantibody screening in the diagnosis of neurological autoimmune diseases. Paper presented at: 2nd International Conference on Antibodies and Therapeutics; July 11-12, 2016; Philadelphia, PA. https://www.longdom.org/proceedings/multiparameter-autoantibody-screening-in-the-diagnosis-of-neurological-autoimmune-diseases-6855.html **7.** Sato DK, Callegaro D, Lana-Peixoto MA, et al. Distinction between MOG antibody-positive and AQP4 antibody-positive NMO spectrum disorders. *Neurology.* 2014;82(6):474-481. doi:10.1212/WNL.0000000000000101 **8.** Pröbstel AK, Rudolf G, Dornmair K, et al. Anti-MOG antibodies are present in a subgroup of patients with a neuromyelitis optica phenotype. *J Neuroinflammation.* 2015;12:46. doi:10.1186/s12974-015-0256-1 **9.** Zouvelou V, Zisimopoulou P, Rentzos M, et al. Double seronegative myasthenia gravis with anti-LRP4 antibodies. *Neuromuscul Disord.* 2013;23(7):568-570. doi:10.1016/j.nmd.2013.03.013 **10.** Rivner MH, Quarles BM, Pan JX, et al. Clinical features of LRP4/agrin-antibody-positive myasthenia gravis: a multicenter study. *Muscle Nerve.* 2020;62(3):333-343. doi:10.1002/mus.26985 **11.** Phillips WD, Vincent A. Pathogenesis of myasthenia gravis: update on disease types, models, and mechanisms. *F1000Res.* 2016;5(F1000 Faculty Rev):1513. doi:10.12688/f1000research.8206.1 **12.** Dalmau J. National Organization for Rare Disorders. NORD website. Rare disease database: Paraneoplastic neurological syndromes. Updated 2016. Accessed October 20, 2021. https://rarediseases.org/rare-diseases/paraneoplastic-neurologic-syndromes/ **13.** Foundation for Peripheral Neuropathy. Symptoms. Accessed September 1, 2022. https://www.foundationforpn.org/symptoms/

QuestDiagnostics.com