MAYO CLINIC LABORATORIES

Test Definition: NMOFS

Neuromyelitis Optica (NMO)/Aquaporin-4-IgG Fluorescence-Activated Cell Sorting (FACS) Assay, Serum

Overview

Useful For

Diagnosis of a neuromyelitis optica spectrum disorder (NMOSD)

Diagnosis of autoimmune AQP4 channelopathy

Diagnosis of neuromyelitis optica (NMO)

Distinguishing NMOSD from multiple sclerosis early in the course of disease

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
NMOTS	NMO/AQP4 FACS Titer, S	No	No

Testing Algorithm

When the results of this assay require further evaluation, NMOTS / Neuromyelitis Optica (NMO)/Aquaporin-4-IgG Fluorescence-Activated Cell Sorting (FACS) Titer Assay, Serum will be performed at an additional charge.

Method Name

Flow Cytometry

NY State Available

Yes

Specimen

Specimen Type

Serum

Specimen Required

Collection Container/Tube:
Preferred: Red top
Acceptable: Serum gel
Submission Container/Tube: Plastic vial
Specimen Volume: 3 mL
Collection Instructions: Centrifuge and aliquot serum into a plastic vial.

Forms

If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:
-General Request (T239)
-Neurology Specialty Testing Client Test Request (T732)

Specimen Minimum Volume

2 mL

Reject Due To

Gross hemolysis	OK
Gross lipemia	OK
Gross icterus	OK

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum	Refrigerated (preferred)	28 days	
	Ambient	72 hours	
	Frozen	28 days	

Clinical & Interpretive

Clinical Information

Neuromyelitis optica (NMO), sometimes called Devic disease or opticospinal multiple sclerosis [MS]) is a severe, relapsing, autoimmune, inflammatory and demyelinating central nervous system disease that predominantly affects optic nerves and spinal cord.(1) The disorder is now recognized as a spectrum of autoimmunity (termed NMO spectrum disorders [NMOSD]) targeting the astrocytic water channel aquaporin-4 (AQP4).(1,2) Brain lesions are observed in >60% of patients with NMOSD and approximately 10% will be MS-like.(3) Children tend to have greater brain involvement than adults and brain lesions are more symptomatic than is typical for adult patients.(4) Extensive cerebral white matter signal abnormalities are sometimes encountered, most commonly in children, and are sometimes associated with encephalopathy. Circumventricular organs (CVO; eg, area postrema) are preferentially involved. Symptoms and signs attributable to area postrema involvement include intractable hiccups, nausea and vomiting, and these may occur in isolation, herald the onset of NMO or occur in association with the more classical optic neuritis or Longitudinally Extensive Transverse Myelitis (LETM).(5) Magnetic resonance imaging typically reveals large inflammatory spinal cord lesions involving 3 or more vertebral segments. During acute attacks, the cerebrospinal fluid contains inflammatory cells, but usually lacks evidence of intrathecal IgG synthesis. The clinical course is characterized by relapses of optic neuritis or transverse myelitis, or both. Many patients with NMOSD are misdiagnosed as having MS. Importantly, the prognosis and optimal treatments for the 2 diseases differ. NMOSD typically has a worse natural history than MS, with frequent and early relapses. NMOSD attacks are often severe resulting in a rapid accumulation of disability (blindness and paraplegia). More effective treatments combined with earlier and more accurate diagnosis has led to improved outcomes. Currently,

in the AQP4-IgG era, 5 years after onset, approximately 30% of NMO patients will require a cane to walk and 10% will be wheelchair bound. Treatments for NMOSD include corticosteroids and plasmapheresis for acute attacks and mycophenolate mofetil, azathioprine, and rituximab for relapse prevention. Beta-interferon, a treatment promoted for MS, exacerbates NMOSD. Therefore, early diagnosis and initiation of NMO-appropriate immunosuppressant treatment is important to optimize the clinical outcome by preventing further attacks. Skeletal muscle abnormalities with hyperCKemia have been reported in a few NMOSD patients. Recent reports indicate focal retinal vascular attenuation, inner nuclear layer thickening and microcystic edema in some NMO patients. The sensitivity and specificity of Fluorescence-Activated Cell Sorting (FACS) assay for NMO is >80% and >99%, respectively.

Detection of NMO/APQ4-IgG allows distinction of NMOSD from MS and is indicative of a relapsing disease, mandating initiation of immunosuppression, even after the first attack, thereby reducing attack frequency and disability in the future.

Reference Values

Negative

Interpretation

A positive value is consistent with a neuromyelitis optica spectrum disorder (NMOSD) and justifies initiation of appropriate immunosuppressive therapy at the earliest possible time. This allows early initiation and maintenance of optimal therapy. Recommend follow-up in 3 to 6 months if NMOSD is suspected.

This autoantibody is not found in healthy subjects.

Cautions

AQP4-IgG antibodies may drop below detectable levels in setting of therapies for acute attack (IV methylprednisolone or plasmapheresis) or attack prevention (immunosuppressants).

Supportive Data

An international collaborative group (Mayo Clinic, Oxford University, and McGill University) compared sensitivity and specificity of AQP4 FACS assays to other assay types including fixed, permeabilized cell-based assays (CBA, observer-scored immunofluorescence, Euroimmun), tissue-based immunofluorescence (IF), ELISA, and immunoprecipitation assay (IPA) in a blinded fashion among 60 neuromyelitis optica spectrum disorder (NMOSD) cases and 86 control subjects. Clinical sensitivity of AQP4 FACS was superior to the other assay types. Sensitivities were: AQP4 FACS (M23 isoform), 77%, AQP4-CBA (M1 isoform), 73%; M1-AQP4-ELISA, 60%; IPA, 53%; tissue-based IF, 48%. Specificities were 100% for all assay types, except the Mayo Clinic IPA (97%).(6)

In 2014, a systematic comparison of AQP4-IgG assays, in a clinical service setting, confirmed superiority of FACS assays over ELISA. Higher-order arrays of M23-AQP4 (M23-AQP4-FACS) and M1-AQP4-ELISA were associated with false-positive results. Overall, M1-AQP4-FACS was 83% sensitive for NMO compared with 75% for M23-AQP4-FACS, 75% for M1-AQP4-CBA and 58% for M1-AQP4-ELISA. Assays specificities for NMO were: M1-AQP4-FACS, 100%, M1-AQP4-CBA, 100%, M1-AQP4- ELISA, 99%; and M23-AQP4- FACS, 95%.(7)

AQP4 FACS analysis was done for serum samples from 36 random patients with a diagnosis of NMO. All samples were tested with our validated AQP4 CBA assay. Thirty samples (83.33%) were positive by FACS and 29 samples (80.55%) were

positive by CBA. All 6 samples that were negative by FACS also tested negative by CBA.

To measure the specificity, AQP4 FACS analysis was done for CSF of 338 non-NMO(SD) patients. None of the samples were positive by this assay (specificity=100%).

Clinical Reference

1. Wingerchuk DM, Lennon VA, Lucchinetti CF, et al: The spectrum of neuromyelitis optica. Lancet Neurol 2007;6:805-815
2. Lennon VA, Wingerchuk DM, Kryzer TJ, et al: A serum autoantibody marker of neuromyelitis optica; distinction from multiple sclerosis. Lancet 2004;364:2106-2112
3. Pittock SJ, Weinshenker BG, Lucchinetti CF, et al: Neuromyelitis optica brain lesions localized at sites of high aquaporin 4 expression. Arch Neurol 2006 Jul;63(7):964-968
4. McKeon A, Lennon VA, Lotze T, et al: CNS aquaporin-4 autoimmunity in children. Neurology 2008 Jul 8;71(2):93-100
5. Apiwattanakul M, Popescu BF, Matiello M, et al: Intractable vomiting as the initial presentation of NMO. Ann Neurol 2010 Nov;68(5):757-761
6. Waters P, McKeon A, Leite MI, et al: Multicentre comparison of aquaporin-4 IgG assays in NMO spectrum disorders. Neurology 2012;78:665-671
7. Fryer JP, Lennon VA, Pittock SJ, et al: AQP4 autoantibody assay performance in clinical laboratory service. Neurol Neuroimmunol Neuroinflammation 2014;1:e11

Performance

Method Description

NMO-IgG Fluorescence-Activated Cell Sorting Assay (FACS)
Human embryonic kidney cells (HEK 293) are transfected transiently with a plasmid (pIRES2- *Aequorea coerulescens* green fluorescent protein [AcGFP]) encoding both green fluorescent protein (AcGFP) and AQP4-M1. After 36 hours, a mixed population of cells (transfected expressing AQP4 on the surface and AcGFP in the cytoplasm and nontransfected lacking AQP4 and AcGFP) are lifted and resuspended in live cell-binding buffer. Patient serum is then added to cells at a 1 in 5 screening dilution. After incubation and washing, the cells are resuspended in secondary antibody (AlexaFluor 647-conjugated goat antihuman IgG; 1:2000 in LCBB), held on ice, washed, fixed with 4% paraformaldehyde, and analyzed by flow cytometry (BD FACSCanto; Becton, Dickinson and Co). Two populations are gated on the basis of AcGFP expression: positive (high AQP4 expression) and negative (low or no AQP4 expression). The median Alexafluor 647 fluorescence intensity (MFI) for the AcGFP-positive population indicates relative abundance of human IgG potentially bound to AQP4 surface epitopes; MFI for the GFP-negative population indicated nonspecifically-bound IgG. The IgG binding index is calculated as the ratio of the average MFI for duplicate aliquots of each cell population (MFI GFP positive/MFI GFP negative).We established conservative cutoff IgG binding index values of 2.00 for M1-FACS.

If FACS assay is positive at screening dilution, FACS Titer Assay is performed at an additional charge. The patient serum is titrated to endpoint. The dilution where the IgG binding index is greater than or equal to 2, is considered the endpoint dilution. If a patient is positive at a 1:5 dilution, but negative at 1:10 dilution, the endpoint will be reported as 5.

Test Definition: NMOFS

Neuromyelitis Optica (NMO)/Aquaporin-4-IgG
Fluorescence-Activated Cell Sorting (FACS)
Assay, Serum

PDF Report
No

Day(s) Performed
Monday, Tuesday, Thursday

Report Available
5 to 8 days

Specimen Retention Time
28 days

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Main Campus

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification
This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
86053
86053-titer (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
NMOFS	NMO/AQP4 FACS, S	43638-6

Result ID	Test Result Name	Result LOINC® Value
38324	NMO/AQP4 FACS, S	43638-6