MAYO CLINIC LABORATORIES

Test Definition: NMOFC

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

Overview

Useful For

Diagnosis of a neuromyelitis optica spectrum disorder (NMOSD)

Diagnosis of autoimmune AQP4 channelopathy

Distinguishing NMOSD from multiple sclerosis early in the course of disease

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
NMOTC	NMO/AQP4 FACS Titer, CSF	No	No

Testing Algorithm

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

Method Name

Flow Cytometry

NY State Available

Yes

Specimen

Specimen Type

CSF

Necessary Information

Include relevant clinical information, name, phone number, mailing address, and e-mail address (if applicable) of ordering physician.

Specimen Required

Collection Container/Tube: Sterile vial

Specimen Volume: 3 mL

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
CSF	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 hyerCKemia 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.

Detection of AQP4-IgG by NMO/AQP4 FACS in cerebrospinal fluid (CSF) allows distinction from MS and is indicative of an NMOSD.

Though serum is optimal for AQP4-IgG testing, occasionally physicians submit only CSF for testing. A previous study, based on our first-generation indirect immunofluorescence assay compared the frequencies of AQP4-IgG in serum and CSF. The positivity rate was greater for serum alone than for CSF alone.(6) However, testing of CSF was helpful when the serum was negative. Detection of AQP4-IgG in CSF allowed unambiguous distinction of NMO from MS. CSF testing offered the additional advantage of generally lacking the nonorgan-specific IgG autoantibodies (eg, antinuclear, antimitochondrial, and smooth muscle) that are common in serum of patients with NMO and also with classic paraneoplastic autoimmune disorders. Recent AQP4 FACS analysis of paired CSF and serum samples from 66 patients submitted for AQP4-IgG testing reveals a slightly better detection rate in serum (n=59) compared with CSF (n=55). All 7 patients who tested negative in serum also tested negative in CSF.

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.

This autoantibody is not found in healthy subjects.

Cautions
A negative result does not exclude a diagnosis of neuromyelitis optica spectrum disorder (NMOSD). Recommend serum be tested if CSF tests negative and neuromyelitis optica spectrum disorder is suspected. 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
Recent AQP4 FACS analysis of paired cerebrospinal fluid (CSF) and serum samples from 66 patients submitted for AQP4-IgG testing reveals a slightly better detection rate in serum (n=59) compared with CSF (n=55). All 7 patients who tested negative in serum also tested negative in CSF.

AQP4 FACS analysis was done for CSF samples from 26 random patients with a diagnosis of NMO. All samples except 1 were tested with our validated AQP4 cell-binding assay (CBA). A total of 18 samples (69.23%) were positive by FACS, while only 14 samples (56%) were positive by CBA. All 8 samples that were negative by FACS also tested negative by CBA.

To measure the specificity, AQP4 FACS analysis was done for CSF of 27 patients with the diagnosis of normal pressure hydrocephalus. 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. McKeon A, Pittock SJ, Lennon VA: CSF complements serum for evaluating paraneoplastic antibodies and NMO-IgG. Neurology 2011 Mar 22;76(12):1108-1110

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 (GFP) and AQP4-M1. After 36 hours, the mixed population of cells (transfected expressing AQP4 on the surface and GFP in the cytoplasm and nontransfected lacking AQP4 and GFP) are harvested with short exposure to trypsin. Patient cerebrospinal fluid (CSF) is then added to cells at a 1 in 2 screening dilution. After incubation and washing, the cells are resuspended with AlexaFluor 647-conjugated goat antihuman IgG-gamma specific secondary antibody (Southern Biotech catalog No. 2040-31, 1:2000 in LCBB), held on ice, washed, fixed with 4% paraformaldehyde, and examined with flow cytometry (BD FACSCanto; Becton, Dickinson and Co). Two populations are gated on the basis of GFP 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 was 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 CSF 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 1:2 dilution, but negative at 1:4, the endpoint will be reported as 2.

PDF Report

No

Day(s) Performed
Monday, Tuesday, Thursday

Report Available
5 to 8 days

Specimen Retention Time
2 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
NMOFC	NMO/AQP4 FACS, CSF	46718-3

Result ID	Test Result Name	Result LOINC® Value
38325	NMO/AQP4-IgG FACS, CSF	46718-3