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

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Reporting Name</th>
<th>Available Separately</th>
<th>Always Performed</th>
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<tbody>
<tr>
<td>NMOTS</td>
<td>NMO/AQP4 FACS Titer, S</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

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

Container/Tube:

Preferred: Red top

Acceptable: Serum gel

Specimen Volume: 3 mL

Forms
If not ordering electronically, complete, print, and send a Neurology Specialty Testing Client Test Request (T732) with the specimen.

Specimen Minimum Volume
2 mL
Test Definition: NMOFS
NMO/AQP4 FACS, S

Reject Due To

<table>
<thead>
<tr>
<th>Gross hemolysis</th>
<th>OK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross lipemia</td>
<td>OK</td>
</tr>
<tr>
<td>Gross icterus</td>
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</table>

Specimen Stability Information

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
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<tbody>
<tr>
<td>Serum</td>
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<tr>
<td>Frozen</td>
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</tr>
<tr>
<td>Ambient</td>
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<td>72 hours</td>
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Clinical and 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.(1) 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/AQP4-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.
Test Definition: NMOFS

NMO/AQP4 FACS, S

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


**Performance**

**Method Description**

NMO-IgG Fluorescence-Activated Cell Sorting Assay (FACS)

Human embryonic kidney cells (HEK 293) are transfected transiently with a plasmid (pIRES2- \textit{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.

**PDF Report**

No

**Day(s) and Time(s) Test Performed**

Monday, Tuesday, Thursday; 6 p.m.

**Analytic Time**

5 days

**Maximum Laboratory Time**

8 days

**Specimen Retention Time**

28 days

**Performing Laboratory Location**

Rochester

**Fees and 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 Regional Manager. 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. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information

86255

86256-NMO/AQP4-IgG FACS titer (if appropriate)

LOINC® Information

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<td>NMO/AQP4 FACS, S</td>
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