

**Overview**
**Useful For**

Evaluating patients with suspected autoimmune myelopathy, myelitis, paraneoplastic myelopathy using serum specimens

**Profile Information**

Test ID	Reporting Name	Available Separately	Always Performed
MSI1	Autoimmune Myelopathy Interp, S	No	Yes
AMPHS	Amphiphysin Ab, S	No	Yes
AGN1S	Anti-Glial Nuclear Ab, Type 1	No	Yes
ANN1S	Anti-Neuronal Nuclear Ab, Type 1	No	Yes
ANN2S	Anti-Neuronal Nuclear Ab, Type 2	No	Yes
ANN3S	Anti-Neuronal Nuclear Ab, Type 3	No	Yes
CRMWS	CRMP-5-IgG Western Blot, S	Yes	Yes
CRMS	CRMP-5-IgG, S	No	Yes
DPPIS	DPPX Ab IFA, S	No	Yes
GD65S	GAD65 Ab Assay, S	Yes	Yes
GFAIS	GFAP IFA, S	No	Yes
GL1IS	mGluR1 Ab IFA, S	No	Yes
MOGFS	MOG FACS, S	Yes	Yes
NIFIS	NIF IFA, S	No	Yes
NMOFS	NMO/AQP4 FACS, S	Yes	Yes
CCN	N-Type Calcium Channel Ab	No	Yes
CCPQ	P/Q-Type Calcium Channel Ab	No	Yes
PCABP	Purkinje Cell Cytoplasmic Ab Type 1	No	Yes
PCAB2	Purkinje Cell Cytoplasmic Ab Type 2	No	Yes
PCATR	Purkinje Cell Cytoplasmic Ab Type Tr	No	Yes

**Reflex Tests**

Test ID	Reporting Name	Available Separately	Always Performed
AGNBS	AGNA-1 Immunoblot, S	No	No
AINCS	Alpha Internexin CBA, S	No	No
AMPBS	AMPA-R Ab CBA, S	No	No
AMPIS	AMPA-R Ab IF Titer Assay, S	No	No
AMIBS	Amphiphysin Immunoblot, S	No	No
AN1BS	ANNA-1 Immunoblot, S	No	No
AN2BS	ANNA-2 Immunoblot, S	No	No
DPPCS	DPPX Ab CBA, S	No	No
DPPTS	DPPX Ab IFA Titer, S	No	No
GABCS	GABA-B-R Ab CBA, S	No	No
GABIS	GABA-B-R Ab IF Titer Assay, S	No	No
GFACS	GFAP CBA, S	No	No
GFATS	GFAP IFA Titer, S	No	No
GL1CS	mGluR1 Ab CBA, S	No	No
GL1TS	mGluR1 Ab IFA Titer, S	No	No
MOGTS	MOG FACS Titer, S	No	No
NFHCS	NIF Heavy Chain CBA, S	No	No
NIFTS	NIF IFA Titer, S	No	No
NFLCS	NIF Light Chain CBA, S	No	No
NMDCS	NMDA-R Ab CBA, S	No	No
NMDIS	NMDA-R Ab IF Titer Assay, S	No	No
NMOTS	NMO/AQP4 FACS Titer, S	No	No
PC1BS	PCA-1 Immunoblot, S	No	No
PCTBS	PCA-Tr Immunoblot, S	No	No

### Testing Algorithm

If indirect immunofluorescence assay (IFA) patterns suggest AGNA-1 antibody, then AGNA-1 Immunoblot is performed at an additional charge.

If IFA patterns suggest amphiphysin antibody, then amphiphysin immunoblot is performed at an additional charge.

If IFA patterns suggest ANNA-1 antibody, then ANNA-1 immunoblot is performed at an additional charge.

If IFA patterns suggest ANNA-2 antibody, then ANNA-2 immunoblot is performed at an additional charge.

If IFA patterns suggest PCA-1 antibody, then PCA-1 immunoblot is performed at an additional charge.

If IFA patterns suggest PCA-Tr antibody, then PCA-Tr immunoblot is performed at an additional charge.

If IFA pattern suggests NMDA-receptor antibody, then NMDA- receptor R antibody cell-binding assay (CBA) and NMDA- receptor R titer are performed at an additional charge.

If IFA pattern suggests AMPA- receptor antibody, then AMPA- receptor antibody CBA and AMPA- receptor titer are performed at an additional charge.

If IFA pattern suggests GABA-B- receptor antibody, then GABA-B- receptor antibody CBA and GABA-B- receptor titer are performed at an additional charge.

If IFA pattern suggests DPPX antibody, then DPPX antibody CBA and DPPX IFA titer are performed at an additional charge.

If IFA pattern suggests mGluR1 antibody, then mGluR1 antibody CBA and mGluR1 IFA titer are performed at an additional charge.

If IFA pattern suggests GFAP antibody, then GFAP antibody CBA and GFAP IFA titer are performed at an additional charge.

If NMO/AQP4-IgG FACS screen assay requires further investigation, then NMO/AQP4-IgG FACS titration assay is performed at an additional charge.

If MOG FACS screen assay requires further investigation, then MOG FACS titration assay is performed at an additional charge.

If IFA pattern suggests NIF antibody, then alpha internexin CBA, NIF heavy chain CBA, NIF light chain CBA, and NIF titer are performed at an additional charge.

See [Autoimmune Myelopathy Evaluation Algorithm-Serum](#) in Special Instructions.

## Special Instructions

- [Autoimmune Myelopathy Evaluation Algorithm-Serum](#)

## Method Name

AGN1S, AMPHS, AMPIS, ANN1S, ANN2S, ANN3S, CRMS, DPPIS, DPPTS, GABIS, GFAIS, GFATS, GL1IS, GL1TS, NIFIS, NIFTS, NMDIS, PCAB2, PCABP, PCATR: Indirect Immunofluorescence Assay (IFA)

CCN, CCPQ, GD65S: Radioimmunoassay (RIA)

CRMWS: Western Blot (WB)

AGNBS, AMIBS, AN1BS, AN2BS, PC1BS, PCTBS: Immunoblot (IB)

MOGFS, MOGTS, NMOFS, NMOTS: Flow Cytometry (FACS)

AINCS, AMPCS, DPPCS, GABCS, GFACS, GL1CS, NFHCS, NFLCS, NMDCS: Cell-Binding Assay (CBA)

## NY State Available

Yes

## Specimen

### Specimen Type

Serum

### Necessary Information

Provide the following information:

- Relevant clinical information
- Ordering provider name, phone number, mailing address, and e-mail address

### Specimen Required

#### Patient Preparation:

1. For optimal antibody detection, specimen collection is recommended prior to initiation of immunosuppressant medication.
2. This test should not be requested in patients who have recently received radioisotopes, therapeutically or diagnostically, because of potential assay interference. The specific waiting period before specimen collection will depend on the isotope administered, the dose given, and the clearance rate in the individual patient. Specimens will be screened for radioactivity prior to analysis. Radioactive specimens received in the laboratory will be held 1 week and assayed if sufficiently decayed, or canceled if radioactivity remains.

#### Container/Tube:

**Preferred:** Red top

**Acceptable:** Serum gel

**Specimen Volume:** 4 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

#### Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject
Gross icterus	Reject

#### Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum	Refrigerated (preferred)	28 days	

Specimen Type	Temperature	Time	Special Container
	Frozen	28 days	
	Ambient	72 hours	

## Clinical and Interpretive

### Clinical Information

Patients with autoimmune myelopathy present with subacute onset and rapid progression of spinal cord symptoms with one or more of the following: weakness, gait difficulties, loss of sensation, neuropathic pain, and bowel and bladder dysfunction. Clinical history and examination, spinal cord magnetic resonance imaging, and cerebrospinal fluid (CSF) testing may provide clues to an autoimmune diagnosis. Autoimmune myelopathy evaluation of both serum and CSF can assist in the diagnosis (paraneoplastic or idiopathic autoimmune) and aid distinction from other causes of myelopathy (multiple sclerosis, sarcoidosis, vascular disease). Early testing may assist in early diagnosis of occult cancer, prompt initiation of immune therapies, or both.

### Reference Values

Test ID	Reporting name	Methodology	Reference value
MSI1	Autoimmune Myelopathy Interp, S		
AMPHS	Amphiphysin Ab, S	Indirect immunofluorescence assay (IFA)	<1:240
AGN1S	Anti-Glial Nuclear Ab, Type 1	IFA	<1:240
ANN1S	ANNA-1, S	IFA	<1:240
ANN2S	ANNA-2, S	IFA	<1:240
ANN3S	ANNA-3, S	IFA	<1:240
CRMWS	CRMP-5-IgG Western Blot, S	Western blot	Negative
CRMS	CRMP-5-IgG, S	IFA	<1:240
DPPIS	DPPX Ab IFA, S	IFA	Negative
GD65S	GAD65 Ab Assay, S	Radioimmunoassay (RIA)	< or =0.02 nmol/L Reference values apply to all ages.
GFAIS	GFAP IFA, S	IFA	Negative
GL1IS	mGluR1 Ab IFA, S	IFA	Negative
MOGFS	MOG FACS, S	Flow cytometry	Negative
NIFIS	NIF IFA, S	IFA	Negative
NMOFS	NMO/AQP4 FACS, S	Flow cytometry	Negative

CCN	N-Type Calcium Channel Ab	RIA	< or = 0.03 nmol/L
CCPQ	P/Q-Type Calcium Channel Ab	RIA	< or =0.02 nmol/L
PCABP	PCA-1, S	IFA	<1:240
PCAB2	PCA-2, S	IFA	<1:240
PCATR	PCA-Tr, S	IFA	<1:240

**Reflex Information**

Test ID	Reporting name	Methodology	Reference value
AGNBS	AGNA-1 Immunoblot, S	Immunoblot (IB)	Negative
AINCS	Alpha Internexin CBA, S	Cell-binding assay (CBA)	Negative
AMPCS	AMPA-R Ab CBA, S	CBA	Negative
AMPIS	AMPA-R Ab IF Titer Assay, S	IFA	<1:120
AMIBS	Amphiphysin Immunoblot, S	IB	Negative
AN1BS	ANNA-1 Immunoblot, S	IB	Negative
AN2BS	ANNA-2 Immunoblot, S	IB	Negative
DPPCS	DPPX Ab CBA, S	CBA	Negative
DPPTS	DPPX Ab IFA Titer, S	IFA	<1:240
GABCS	GABA-B-R Ab CBA, S	CBA	Negative
GABIS	GABA-B-R Ab IF Titer Assay, S	IFA	<1:120
GFACS	GFAP CBA, S	CBA	Negative
GFATS	GFAP IFA Titer, S	IFA	<1:240
GL1CS	mGluR1 Ab CBA, S	CBA	Negative
GL1TS	mGluR1 Ab IFA Titer, S	IFA	<1:240
MOGTS	MOG FACS Titer, S	Flow cytometry	<1:20
NFHCS	NIF Heavy Chain CBA, S	CBA	Negative
NIFTS	NIF IFA Titer, S	IFA	<1:240
NFLCS	NIF Light Chain CBA, S	CBA	Negative
NMDCS	NMDA-R Ab CBA, S	CBA	Negative
NMDIS	NMDA-R Ab IF Titer Assay, S	IFA	<1:120
NMOTS	NMO/AQP4 FACS Titer, S	Flow cytometry	<1:5
PC1BS	PCA-1 Immunoblot, S	IB	Negative
PCTBS	PCA-Tr Immunoblot, S	IB	Negative

Neuron-restricted patterns of IgG staining that do not fulfill criteria for ANNA-1, ANNA-2, CRMP-5-IgG, PCA-1, PCA-2, or PCA-Tr may be reported as "unclassified anti-neuronal IgG." Complex patterns that include nonneuronal

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elements may be reported as "uninterpretable."

## Interpretation

A positive result is consistent with a diagnosis of autoimmune myelopathy in the appropriate clinical context.

## Cautions

Negative results do not exclude a diagnosis of autoimmune myelopathy.

## Clinical Reference

1. Dubey D, Pittock SJ, Krecke KN: Clinical, Radiologic, and Prognostic Features of Myelitis Associated With Myelin Oligodendrocyte Glycoprotein Autoantibody. *JAMA Neurol* 2018 Dec 21. doi: 10.1001/jamaneurol.2018.4053. Epub ahead of print
2. Zalewski NL, Flanagan EP: Autoimmune and Paraneoplastic Myelopathies. *Semin Neurol* 2018 Jun;38(3):278-289
3. Flanagan EP, Hinson SR, Lennon VA: Glial fibrillary acidic protein immunoglobulin G as biomarker of autoimmune astrocytopathy: Analysis of 102 patients. *Ann Neurol* 2017;81:298-309
4. Keegan BM, Pittock SJ, Lennon VA: Autoimmune myelopathy associated with collapsin response-mediator protein-5 immunoglobulin G. *Ann Neurol* 2008;63:531-534
5. Weinshenker BG, Wingerchuk DM, Vukusic S: Neuromyelitis optica IgG predicts relapse after longitudinally extensive transverse myelitis. *Ann Neurol* 2006;59:566-569

## Performance

### Method Description

Indirect Immunofluorescence Assay:

Before testing, patient's serum is preabsorbed with liver powder to remove nonorgan-specific autoantibodies. After applying to a composite substrate of frozen mouse tissues (brain, kidney, and gut) and washing, fluorescein-conjugated goat-antihuman IgG is applied to detect the distribution and pattern of patient IgG binding.(Pittock SJ, Kryzer TJ, Lennon VA: Paraneoplastic antibodies coexist and predict cancer, not neurological syndrome. *Ann Neurol* 2004;56:715-719; Honorat JA, Komorowski L, Josephs KA, et al: IgLON5 antibody: neurological accompaniments and outcomes in 20 patients. *Neurol Neuroimmunol Neuroinflamm* 2017 Jul 18;4(5):e385. doi: 10.1212/NXI.0000000000000385)

Radioimmunoassay:

Duplicate aliquots of patient specimen are incubated with I(125)-labeled antigen. Immune complexes, formed by adding secondary (goat)-antihuman immunoglobulin, are pelleted by centrifugation and washed. Gamma emission from the washed pellet is counted, and mean counts per minute (cpm) are compared with results yielded by high-positive and -negative control sera. Specimen yielding cpm higher than the background cpm yielded by normal human specimen are retested to confirm positivity and titrated as necessary to obtain a value in the linear range of the assay. The antigen binding capacity (nmol per liter) is calculated from the cpm precipitated at a dilution yielding a linear range value.(Griesmann GE, Kryzer TJ, Lennon VA: Autoantibody profiles of myasthenia gravis and Lambert-Eaton myasthenic syndrome. In *Manual of Clinical and Laboratory Immunology*. Sixth edition. Edited by NR Rose, RG Hamilton, et al. ASM Press, 2002, pp 1005-1012; Walikonis JE, Lennon VA: Radioimmunoassay for glutamic acid decarboxylase [GAD65] autoantibodies as a diagnostic aid for stiff-man syndrome and a correlate of

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susceptibility to type1 diabetes mellitus. Mayo Clin Proc 1998;73[12]:1161-1166; Jones AL, Flanagan EP, Pittock SJ, et al: Responses to and Outcomes of Treatment of Autoimmune Cerebellar Ataxia in Adults. JAMA Neurol 2015 Nov;72[11]:1304-1312 doi: 10.1001/jamaneurol.2015.2378)

**Western Blot:**

Neuronal antigens extracted aqueously from adult rat cerebellum, full-length recombinant human collapsin response-mediator protein-5 (CRMP-5), or full-length recombinant human amphiphysin protein is denatured, reduced, and separated by electrophoresis on 10% polyacrylamide gel. IgG is detected autoradiographically by enhanced chemiluminescence (Yu Z, Kryzer TJ, Griesmann GE, et al: CRMP-5 neuronal autoantibody: marker of lung cancer and thymoma-related autoimmunity. Ann Neurol 2001;49[2]:145-154; Dubey D, Jitrapaikulsan J, Bi H, et al: Amphiphysin-IgG autoimmune neuropathy: A recognizable clinicopathologic syndrome. Neurology 2019 Oct 17 pii: 10.1212/WNL.0000000000008472. doi: 10.1212/WNL.0000000000008472)

**Immunoblot:**

All steps are performed at room temperature (18-28 degrees C) utilizing the EUROBlot One instrument. Diluted patient specimen (1:12.5) is added to test strips (strips containing recombinant antigen manufactured and purified using biochemical methods) in individual channels and incubated for 30 minutes. Positive specimens will bind to the purified recombinant antigen and negative specimens will not bind. Strips are washed to remove unbound antibodies and then incubated with anti-human IgG antibodies (alkaline phosphatase-labelled) for 30 minutes. The strips are again washed to remove unbound anti-human IgG antibodies and nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolylphosphate (NBT/BCIP) substrate is added. Alkaline phosphatase enzyme converts the soluble substrate into a colored insoluble product on the membrane to produce a black band. Strips are digitized via picture capture on the EUROBlot One instrument and evaluated with the EUROLineScan software. (O'Connor K, Waters P, Komorowski L, et al: GABAA receptor autoimmunity: A multicenter experience. Neurol Neuroimmunol Neuroinflamm 2019 Apr 4;6[3]:e552 doi: 10.1212/NXI.000000000000552)

**NMO-IgG Fluorescence-Activated Cell Sorting Assay/Flow Cytometry:**

Human embryonic kidney cells (HEK 293) are transfected transiently with a plasmid (pIRES2- *Aequorea coerulea* 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. Cells are incubated with patient serum and an AlexaFluor 647-labeled secondary antibody is added. Two populations are gated on the basis of AcGFP expression: positive (high AQP4 expression) and negative (low or no AQP4 expression). Positivity is based on the ratio (Positive >2.0) of the average MFI of each cell population (MFI GFP positive:MFI GFP negative). (Fryer JP, Lennon VP, Pittock SJ, et al: AQP4 autoantibody assay performance in clinical laboratory service. Neurol Neuroimmunol Neuroinflamm 2014 May 22; 1[1]:e11. doi: 10.1212/NXI.0000000000000011)

**MOG-IgG1 Fluorescence-Activated Cell Sorting Assay (FACS)/Flow Cytometry:**

Human embryonic kidney cells (HEK 293) are transfected transiently with a DNA plasmid that allows coexpression of both a reporter fluorescent protein (green fluorescent protein [AcGFP]) and full-length MOG. After 36 hours, a mixed population of cells (transfected expressing MOG on the surface and AcGFP in the cytoplasm and nontransfected lacking MOG and AcGFP) are lifted and resuspended in live cell-binding buffer. Cells are incubated with patient serum and an AlexaFluor 647 labeled secondary antibody is added. Two populations are gated on the basis of AcGFP expression: positive (high MOG expression) and negative (low or no MOG expression). Positivity is based on the ratio (Positive >2.5) of the average MFI of each cell population (MFI GFP positive:MFI GFP negative). (Fryer JP, Lennon VP, Pittock SJ, et al: AQP4 autoantibody assay performance in clinical laboratory service. Neurol Neuroimmunol Neuroinflamm 2014 May 22; 1[1]:e11. doi: 10.1212/NXI.0000000000000011)



**PDF Report**

No

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

AGN1S, AMPHS, AMPIS, ANN1S, ANN2S, ANN3S, CRMS, DPPIS, DPPTS, GABIS, GFAIS, GFATS, GL1IS, GL1TS, NIFIS, NIFTS, NMDIS, PCAB2, PCABP, PCATR:

Monday through Friday; 5 a.m., 7 a.m., 5 p.m.

Saturday, Sunday; 6 a.m.

CCN, CCPQ:

Monday through Friday; 6 a.m., 8 a.m., 6 p.m.

Saturday, Sunday; 7 a.m.

CRMWS:

Monday through Thursday; 8 a.m.

AGNBS, AMIBS, AN1BS, AN2BS, PC1BS, PCTBS:

Monday through Friday; 6 p.m.

GD65S:

Monday through Friday; 5 a.m., 2 p.m.

Saturday, Sunday; 7 a.m.

MOGFS, MOGTS, NMOFS, NMOTS:

Monday, Tuesday, Thursday; 6 p.m.

AMPCS, DPPCS, GABCS, NMDCS:

Monday through Friday, Sunday; 10 p.m.

GL1CS:

Monday, Thursday; 6 p.m.

AINCS, NFHCS, NFLCS:

Tuesday, Thursday; 6 p.m.

GFACS:

Monday, Wednesday, Friday; 6 p.m.

**Analytic Time**

10 days

**Maximum Laboratory Time**

13 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**

83519 x 2

86255 x15

86341

84182

84182-AGNBS (if appropriate)

86255-AINCS (if appropriate)

86255-AMPCS (if appropriate)

86256-AMPIS (if appropriate)

84182-AMIBS (if appropriate)

84182-AN1BS (if appropriate)

84182-AN2BS (if appropriate)

86255-DPPCS (if appropriate)

86256-DPPTS (if appropriate)

86255-GABCS (if appropriate)

86256-GABIS (if appropriate)

86255-GFACS (if appropriate)

86256-GFATS (if appropriate)

86255-GL1CS (if appropriate)

86256-GL1TS (if appropriate)

86256-MOGFS (if appropriate)

86256-MOGTS (if appropriate)

86255-NFHCS (if appropriate)

86256-NIFTS (if appropriate)

86255-NFLCS (if appropriate)

86255-NMDCS (if appropriate)

86256-NMDIS (if appropriate)

86256-NMOFS (if appropriate)

86256-NMOTS (if appropriate)

84182-PC1BS (if appropriate)

84182-PCTBS (if appropriate)

**LOINC® Information**

Test ID	Test Order Name	Order LOINC Value
MAS1	Autoimmune Myelopathy Eval, S	94339-9

Result ID	Test Result Name	Result LOINC Value
80776	ANNA-2, S	94343-1
83137	ANNA-3, S	94344-9
81184	N-Type Calcium Channel Ab	94348-0
81185	P/Q-Type Calcium Channel Ab	94349-8
83077	CRMP-5-IgG, S	94815-8
83107	CRMP-5-IgG Western Blot, S	47401-5
81596	GAD65 Ab Assay, S	94345-6

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Result ID	Test Result Name	Result LOINC Value
83138	PCA-2, S	94351-4
9477	PCA-1, S	94350-6
83076	PCA-Tr, S	94352-2
38324	NMO/AQP4 FACS, S	43638-6
65563	MOG FACS, S	90248-6
64930	DPPX Ab IFA, S	82976-2
64928	mGluR1 Ab IFA, S	94347-2
605127	Autoimmune Myelopathy Interp, S	69048-7
605155	GFAP IFA, S	94346-4
606964	NIF IFA, S	96486-6
89080	AGNA-1, S	94341-5
81722	Amphiphysin Ab, S	94340-7
80150	ANNA-1, S	94342-3
36349	Reflex Added	77202-0