Test Definition: MAC1

Autoimmune Myelopathy Eval, CSF

Overview

Useful For

Evaluating patients with suspected autoimmune myelopathy, myelitis, paraneoplastic myelopathy using spinal fluid specimens

Profile Information

Test ID	Reporting Name	Available Separately	Always Performed
MCI1	Autoimmune Myelopathy Interp, CSF	No	Yes
AMPHC	Amphiphysin Ab, CSF	No	Yes
AGN1C	Anti-Glial Nuclear Ab, Type 1	No	Yes
ANN1C	Anti-Neuronal Nuclear Ab, Type 1	No	Yes
ANN2C	Anti-Neuronal Nuclear Ab, Type 2	No	Yes
ANN3C	Anti-Neuronal Nuclear Ab, Type 3	No	Yes
CRMWC	CRMP-5-IgG Western Blot, CSF	Yes	Yes
CRMC	CRMP-5-IgG, CSF	No	Yes
DPPIC	DPPX Ab IFA, CSF	No	Yes
GD65C	GAD65 Ab Assay, CSF	Yes	Yes
GFAIC	GFAP IFA, CSF	No	Yes
GL1IC	mGluR1 Ab IFA, CSF	No	Yes
NIFIC	NIF IFA, CSF	No	Yes
NMOFC	NMO/AQP4 FACS, CSF	Yes	Yes
PCTRC	Purkinje Cell Cytoplasmc Ab Type Tr	No	Yes
PCA1C	Purkinje Cell Cytoplasmic Ab Type 1	No	Yes
PCA2C	Purkinje Cell Cytoplasmic Ab Type 2	No	Yes

Reflex Tests

Test ID	Reporting Name	Available Separately	Always Performed
AGNBC	AGNA-1 Immunoblot, CSF	No	No
AINCC	Alpha Internexin CBA, CSF	No	No

Test ID	Reporting Name	Available Separately	Always Performed
AMPCC	AMPA-R Ab CBA, CSF	No	No
AMPIC	AMPA-R Ab IF Titer Assay, CSF	No	No
AMIBC	Amphiphysin Immunoblot, CSF	No	No
AN1BC	ANNA-1 Immunoblot, CSF	No	No
AN2BC	ANNA-2 Immunoblot, CSF	No	No
DPPCC	DPPX Ab CBA, CSF	No	No
DPPTC	DPPX Ab IFA Titer, CSF	No	No
GABCC	GABA-B-R Ab CBA, CSF	No	No
GABIC	GABA-B-R Ab IF Titer Assay, CSF	No	No
GFACC	GFAP CBA, CSF	No	No
GFATC	GFAP IFA Titer, CSF	No	No
GL1CC	mGluR1 Ab CBA, CSF	No	No
GL1TC	mGluR1 Ab IFA Titer, CSF	No	No
NFHCC	NIF Heavy Chain CBA, CSF	No	No
NIFTC	NIF IFA Titer, CSF	No	No
NFLCC	NIF Light Chain CBA, CSF	No	No
NMDCC	NMDA-R Ab CBA, CSF	No	No
NMDIC	NMDA-R Ab IF Titer Assay, CSF	No	No
NMOTC	NMO/AQP4 FACS Titer, CSF	No	No
PC1BC	PCA-1 Immunoblot, CSF	No	No
PCTBC	PCA-Tr Immunoblot, CSF	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 suggest NMDA-receptor antibody, then NMDA- receptor antibody cell-binding assay (CBA) and NMDA-

receptor titer are performed at an additional charge.

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

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

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

If IFA pattern suggest mGluR1 antibody, then mGluR1antibody CBA and mGluR1 IFA titer are performed at an additional charge.

If IFA pattern suggest 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 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-Spinal Fluid in Special Instructions.

Special Instructions

- Autoimmune Myelopathy Evaluation Algorithm-Spinal Fluid

Method Name

AGN1C, AMPHC, AMPIC, ANN1C, ANN2C, ANN3C, CRMC, DPPIC, DPPTC, GABIC, GFAIC, GFATC, GL1IC, GL1TC, NIFIC, NIFTC, NMDIC, PCA1C, PCA2C, PCTRC: Indirect Immunofluorescence Assay (IFA)

GD65C: Radioimmunoassay (RIA)

CRMWC: Western Blot (WB)

AGNBC, AMIBC, AN1BC, AN2BC, PC1BC, PCTBC: Immunoblot (IB)

NMOFC, NMOTC: Flow Cytometry (FACS)

AINCC, AMPCC, DPPCC, GABCC, GFACC, GL1CC, NFHCC, NFLCC, NMDCC: Cell Binding Assay (CBA)

NY State Available

Yes

Specimen

Specimen Type

CSF

Necessary Information

Provide the following information:

-Relevant clinical information

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

Specimen Required

Container/Tube: Sterile vial

Preferred: Vial number 1

Acceptable: Any vial

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
CSF	Refrigerated (preferred)	28 days	
	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
MCI1	Autoimmune Myelopathy Interp, CSF		
AMPHC	Amphiphysin Ab, CSF	Indirect immunofluorescence assay (IFA)	<1:2
AGN1C	Anti-Glial Nuclear Ab, Type 1	IFA	<1:2
ANN1C	Anti-Neuronal Nuclear Ab, Type 1	IFA	<1:2
ANN2C	Anti-Neuronal Nuclear Ab, Type 2	IFA	<1:2
ANN3C	Anti-Neuronal Nuclear Ab, Type 3	IFA	<1:2
CRMWC	CRMP-5-IgG Western Blot, CSF	Western blot	Negative
CRMC	CRMP-5-IgG, CSF	IFA	<1:2
DPPIC	DPPX Ab IFA, CSF	IFA	Negative
GD65C	GAD65 Ab Assay, CSF	Radioimmunoassay	< or =0.02 nmol/L Reference values apply to all ages.
GFAIC	GFAP IFA, CSF	IFA	Negative
GL1IC	mGluR1 Ab IFA, CSF	IFA	Negative
NIFIC	NIF IFA, CSF	IFA	Negative
NMOFC	NMO/AQP4 FACS, CSF	Flow cytometry	Negative
PCTRC	Purkinje Cell Cytoplasmic Ab Type Tr	IFA	<1:2
PCA1C	Purkinje Cell Cytoplasmic Ab Type 1	IFA	<1:2
PCA2C	Purkinje Cell Cytoplasmic Ab Type 2	IFA	<1:2

Reflex Information:

Test ID	Reporting name	Methodology	Reference value
AGNBC	AGNA-1 Immunoblot, CSF	Immunoblot (IB)	Negative
AINCC	Alpha Internexin CBA, CSF	Cell-binding assay (CBA)	Negative
AMPCC	AMPA-R Ab CBA, CSF	CBA	Negative
AMPIC	AMPA-R Ab IF Titer Assay, CSF	IFA	<1:2

AMIBC	Amphiphysin Immunoblot, CSF	IB	Negative
AN1BC	ANNA-1 Immunoblot, CSF	IB	Negative
AN2BC	ANNA-2 Immunoblot, CSF	IB	Negative
DPPCC	DPPX Ab CBA, CSF	CBA	Negative
DPPTC	DPPX Ab IFA Titer, CSF	IFA	<1:2
GABCC	GABA-B-R Ab CBA, CSF	CBA	Negative
GABIC	GABA-B-R Ab IF Titer Assay, CSF	IFA	<1:2
GFACC	GFAP CBA, CSF	CBA	Negative
GFATC	GFAP IFA Titer, CSF	IFA	<1:2
GL1CC	mGluR1 Ab CBA, CSF	CBA	Negative
GL1TC	mGluR1 Ab IFA Titer, CSF	IFA	<1:2
NFHCC	NIF Heavy Chain CBA, CSF	CBA	Negative
NIFTC	NIF IFA Titer, CSF	IFA	<1:2
NFLCC	NIF Light Chain CBA, CSF	CBA	Negative
NMDCC	NMDA-R Ab CBA, CSF	CBA	Negative
NMDIC	NMDA-R Ab IF Titer Assay, CSF	IFA	<1:2
NMOTC	NMO/AQP4 FACS Titer, CSF	Flow cytometry	<1:2
PC1BC	PCA-1 Immunoblot, CSF	IB	Negative
PCTBC	PCA-Tr Immunoblot, CSF	IB	Negative

Neuron-restricted patterns of IgG staining that do not fulfill criteria for ANNA-1, ANNA-2, ANNA-3, CRMP-5-IgG, PCA-1, PCA-2, or PCA-Tr may be reported as "unclassified anti-neuronal IgG." Complex patterns that include nonneuronal 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 specimen 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 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, GriesmannGE, et al: CRMP-5 neuronal autoantibody: marker of lung cancer and thymoma-related autoimmunity. Ann Neurol 2001;49[2]:145-154; Dubey D, Jitprapaikulsan 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 specimens (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 produces 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.0000000000000552)

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

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 specimen 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.(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)

If the fluorescence-activated cell sorting (FACS) assay is positive at screening dilution, FACS titer assay is performed at an additional charge. The patient specimen 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

AGN1C, AMPHC, AMPIC, ANN1C, ANN2C, ANN3C, CRMC, DPPIC, DPPTC, GABIC, GFAIC, GFATC, GL1IC, GL1TC, NIFIC, NIFTC, NMDIC, PCA1C, PCA2C, PCTRC:

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

Saturday, Sunday; 6 a.m.

CRMWC:

Monday through Thursday; 8 a.m.

AGNBC, AMIBC, AN1BC, AN2BC, PC1BC, PCTBC:

Monday through Friday; 6 p.m.

GD65C:

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

Saturday, Sunday; 7 a.m.

NMOFC, NMOTC:

Monday, Tuesday, Thursday; 6 p.m.

GFACC:

Monday, Wednesday, Friday; 6 p.m.

AMPCC, DPPCC, GABCC, NMDCC:

Monday through Friday, Sunday; 10 p.m.

AINCC, NFHCC, NFLCC:

Tuesday, Thursday; 6 p.m.

GL1CC:

Monday, Thursday; 6 p.m.

Analytic Time

8 days

Maximum Laboratory Time

11 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

86341

84182

86255 x14

84182-AGNBC (if appropriate)

86255-AINCC (if appropriate)

86255-AMPCC (if appropriate)

86256-AMPIC (if appropriate)

84182-AMIBC (if appropriate)

84182-AN1BC (if appropriate)

84182-AN2BC (if appropriate)

86255-DPPCC (if appropriate)

86256-DPPTC (if appropriate)

86255-GABCC (if appropriate)

86256-GABIC (if appropriate)

86255-GFACC (if appropriate)

86256-GFATC (if appropriate)

86255-GL1CC (if appropriate)

86256-GL1TC (if appropriate)

86255-NFHCC (if appropriate)

86256-NIFTC (if appropriate)

86255-NFLCC (if appropriate)

86255-NMDCC (if appropriate)

86256-NMDIC (if appropriate)

86256-NMOFC (if appropriate)

86256-NMOTC (if appropriate)

84182-PC1BC (if appropriate)

84182-PCTBC (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
MAC1	Autoimmune Myelopathy Eval, CSF	94353-0

Result ID	Test Result Name	Result LOINC Value
38325	NMO/AQP4-IgG FACS, CSF	46718-3
64929	DPPX Ab IFA, CSF	82989-5
64927	mGluR1 Ab IFA, CSF	94361-3
605156	GFAP IFA, CSF	94360-5
605128	Autoimmune Myelopathy Interp, CSF	69048-7
606965	NIF IFA, CSF	In Process
89079	AGNA-1, CSF	94355-5
5906	Amphiphysin Ab, CSF	94354-8
3852	ANNA-1, CSF	94356-3
7472	ANNA-2, CSF	94357-1
21633	ANNA-3, CSF	94358-9
21650	CRMP-5-IgG, CSF	94706-9
3988	PCA-1, CSF	94363-9
21632	PCA-2, CSF	94364-7
21631	PCA-Tr, CSF	94362-1
21747	CRMP-5-IgG Western Blot, CSF	53707-6
21702	GAD65 Ab Assay, CSF	94359-7
36429	Reflex Added	77202-0