

**Overview**
**Useful For**

Evaluating children with autoimmune central nervous system disorders using serum specimens

**Profile Information**

Test ID	Reporting Name	Available Separately	Always Performed
PCSI	Peds Autoimmune CNS Interp, S	No	Yes
ANN1S	Anti-Neuronal Nuclear Ab, Type 1	No	Yes
CS2CS	CASPR2-IgG CBA, S	No	Yes
DPPIS	DPPX Ab IFA, S	No	Yes
GABCS	GABA-B-R Ab CBA, S	No	Yes
GD65S	GAD65 Ab Assay, S	Yes	Yes
GFAIS	GFAP IFA, S	No	Yes
LG1CS	LGI1-IgG CBA, S	No	Yes
GL1IS	mGluR1 Ab IFA, S	No	Yes
MOGFS	MOG FACS, S	Yes	Yes
NMDCS	NMDA-R Ab CBA, S	No	Yes
NMOFS	NMO/AQP4 FACS, S	Yes	Yes
PCATR	Purkinje Cell Cytoplasmic Ab Type Tr	No	Yes

**Reflex Tests**

Test ID	Reporting Name	Available Separately	Always Performed
AGN1S	Anti-Glial Nuclear Ab, Type 1	No	No
AGNBS	AGNA-1 Immunoblot, S	No	No
AMIBS	Amphiphysin Immunoblot, S	No	No
AMPCS	AMPA-R Ab CBA, S	No	No
AMPHS	Amphiphysin Ab, S	No	No
AMPIS	AMPA-R Ab IF Titer Assay, S	No	No
AN1BS	ANNA-1 Immunoblot, S	No	No
AN2BS	ANNA-2 Immunoblot, S	No	No
ANN2S	Anti-Neuronal Nuclear Ab, Type 2	No	No

Test ID	Reporting Name	Available Separately	Always Performed
ANN3S	Anti-Neuronal Nuclear Ab, Type 3	No	No
DPPCS	DPPX Ab CBA, S	No	No
DPPTS	DPPX Ab IFA Titer, 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
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
PCAB2	Purkinje Cell Cytoplasmic Ab Type 2	No	No
PCABP	Purkinje Cell Cytoplasmic Ab Type 1	No	No
PCTBS	PCA-Tr Immunoblot, S	No	No

## Testing Algorithm

If indirect immunofluorescence assay (IFA) patterns suggest antineuronal nuclear antibodies (ANNA)-1 antibody, then ANNA-1 Immunoblot and ANNA-2 Immunoblot are performed at an additional charge.

If IFA patterns suggest ANNA-2 antibody, then ANNA-2 immunoblot, ANNA-1 immunoblot, and ANNA-2 antibody IFA are performed at an additional charge.

If IFA patterns suggest ANNA-3 antibody, then ANNA-3 IFA is performed at an additional charge.

If IFA patterns suggest Purkinje cytoplasmic antibody (PCA)-1 antibody, then PCA-1 immunoblot and PCA-1 IFA are performed at an additional charge.

If IFA patterns suggest PCA-2 antibody, then PCA-2 IFA 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 patterns suggest amphiphysin antibody, then amphiphysin immunoblot and amphiphysin antibody IFA titer are performed at an additional charge.

If IFA pattern suggests N-methyl-D-aspartate receptor (NMDA-R) antibody, then NMDA-R IFA titer is performed at an additional charge.

If IFA pattern suggests alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPA-R) antibody, then AMPA-R antibody cell-binding assay (CBA) and AMPA-R IFA titer are performed at an additional charge.

If IFA pattern suggest gamma-aminobutyric acid B receptor (GABA-B-R) antibody, then GABA-B-R IFA titer is performed at an additional charge.

If IFA pattern suggest dipeptidyl-peptidase-like protein-6 (DPPX) antibody, then DPPX antibody CBA and DPPX IFA titer are performed at an additional charge.

If IFA pattern suggest metabotropic glutamate receptor 1 (mGluR1) antibody, then mGluR1 antibody CBA and mGluR1 IFA titer are performed at an additional charge.

If IFA pattern suggest glial fibrillary acidic protein (GFAP) antibody, then GFAP antibody CBA and GFAP IFA titer are performed at an additional charge.

If neuromyelitis optica/aquaporin-4-IgG (NMO/AQP4-IgG) fluorescence-activated cell sorting (FACS) screen assay requires further investigation, then NMO/AQP4-IgG FACS titration assay is performed at an additional charge.

If myelin oligodendrocyte glycoprotein (MOG) FACS screen assay requires further investigation, then MOG FACS titration assay is performed at an additional charge.

See [Pediatric Autoimmune Central Nervous System Disorders Evaluation Algorithm-Serum](#) in Special Instructions.

### Special Instructions

- [Pediatric Autoimmune Central Nervous System Disorders Evaluation Algorithm-Serum](#)

### Method Name

AMPCS, CS2CS, DPPCS, GABCS, GFACS, GL1CS, LG1CS, NMDCS: Cell-Binding Assay (CBA)

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

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

GD65S: Radioimmunoassay (RIA)

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

### 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 or intravenous immunoglobulin treatment.
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	
	Frozen	28 days	
	Ambient	72 hours	

**Clinical and Interpretive**
**Clinical Information**

Autoimmune encephalitis and myelitis is increasingly recognized as a cause of central nervous system (CNS) disease in children and teens. N-methyl-D-aspartate receptor antibody (NMDA-R) encephalitis and myelin oligodendrocyte glycoprotein (MOG) autoimmunity are most common, though other entities, including aquaporin-4 autoimmunity, contactin-associated protein-like 2 (CASPR2) autoimmunity, autoimmune glial fibrillary acidic protein (GFAP) astrocytopathy, and paraneoplastic encephalomyelopathies, may also occur in children.

**Reference Values**

Test ID	Reporting name	Methodology	Reference value
PCSI	Peds Autoimmune CNS Interp, S	Interpretation	NA
ANN1S	ANNA-1, S	Indirect Immunofluorescence (IFA)	<1:240*
CS2CS	CASPR2-IgG CBA, S	Cell-Binding Assay (CBA)	Negative
DPPIS	DPPX Ab IFA, S	IFA	Negative
GABCS	GABA-B-R Ab CBA, S	CBA	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
LG1CS	LG11-IgG CBA, S	CBA	Negative
GL1IS	mGluR1 Ab IFA, S	IFA	Negative
MOGFS	MOG FACS, S	Flow Cytometry (FACS)	Negative
NMDCS	NMDA-R Ab CBA, S	CBA	Negative
NMOFS	NMO/AQP4 FACS, S	FACS	Negative
PCATR	PCA-Tr, S	IFA	<1:240*

**Reflex Information:**

Test ID	Reporting name	Methodology	Reference value
AGN1S	Anti-Glial Nuclear Ab, Type 1	IFA	<1:240
AGNBS	AGNA-1 Immunoblot, S	Immunoblot (IB)	Negative
AMIBS	Amphiphysin Immunoblot, s	IB	Negative
AMPCS	AMPA-R Ab CBA, S	CBA	Negative
AMPHS	Amphiphysin Ab, S	IFA	<1:240
AMPIS	AMPA-R Ab IF Titer Assay, S	IFA	<1:120
AN1BS	ANNA-1 Immunoblot, S	IB	Negative
AN2BS	ANNA-2 Immunoblot, S	IB	Negative
ANN2S	ANNA-2, S	IFA	<1:240*
ANN3S	ANNA-3, S	IFA	<1:240
DPPCS	DPPX Ab CBA, S	CBA	Negative
DPPTS	DPPX Ab IFA Titer, S	IFA	<1:240
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	FACS	<1:20
NMDIS	NMDA-R Ab IF Titer Assay, S	IFA	<1:120
NMOTS	NMO/AQP4 FACS Titer, S	FACS	<1:5
PC1BS	PCA-1 Immunoblot, S	IB	Negative
PCAB2	PCA-2, S	IFA	<1:240*
PCABP	PCA-1, S	IFA	<1:240*
PCTBS	PCA-Tr Immunoblot, S	IB	Negative

\*Neuron-restricted patterns of IgG staining that do not fulfill criteria for ANNA-1, ANNA-2, 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

This profile is consistent with an autoimmune central nervous system disorder.

## Cautions

Negative results do not exclude a diagnosis of an autoimmune central nervous system disorder.

Intravenous immunoglobulin (IVIg) treatment prior to the serum collection may cause a false-positive result.

## Clinical Reference

1. Dubey D, Pittock SJ, Krecke KN, et al: Clinical, radiologic, and prognostic features of myelitis associated with myelin oligodendrocyte glycoprotein autoantibody. *JAMA Neurol.* 2019 Mar 1;76(3):301-309. doi: 10.1001/jamaneurol.2018.4053
2. McKeon A, Lennon VA, Lotze T, et al: CNS aquaporin-4 autoimmunity in children. *Neurology.* 2008 Jul 8;71(2):93-100
3. Dubey D, Hinson SR, Jolliffe EA, et al: Autoimmune GFAP astrocytopathy: Prospective evaluation of 90 patients in 1 year. *J Neuroimmunol.* 2018 Aug 15;321:157-163
4. Philipps G, Alisanski SB, Pranzatelli M, Clardy SL, Lennon VA, McKeon A: Purkinje cell cytoplasmic antibody type 1 (anti-Yo) autoimmunity in a child with Down syndrome. *JAMA Neurol.* 2014 Mar;71(3):347-349
5. Lopez-Chiriboga AS, Klein C, Zekeridou A, et al: LGI1 and CASPR2 neurological autoimmunity in children. *Ann Neurol.* 2018 Sep;84(3):473-480
6. Lopez-Chiriboga AS, Majed M, Fryer J, et al: Association of MOG-IgG serostatus with relapse after acute disseminated encephalomyelitis and proposed diagnostic criteria for MOG-IgG-associated disorders. *JAMA Neurol.* 2018 Nov 1;75(11):1355-1363
7. Clardy SL, Lennon VA, Dalmau J: Childhood onset of stiff-man syndrome. *JAMA Neurol.* 2013

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Dec;70(12):1531-1536

8. Banwell B, Tenenbaum S, Lennon VA, et al: Neuromyelitis optica-IgG in childhood inflammatory demyelinating CNS disorders. *Neurology*. 2008 Jan 29;70(5):344-352

## 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; Basal E, Zalewski N, Kryzer TJ, et al: Paraneoplastic neuronal intermediate filament autoimmunity. *Neurology*. 2018 Oct 30;91[18]:e1677-e1689)

Radioimmunoassay:

Goat-antihuman IgG and IgM is used as precipitant in all assays. Cation channel protein antigens are solubilized from neuronal or muscle membrane, in nonionic detergent, and complexed with a selective high-affinity ligand labeled with (125)I. (125)I-labelled recombinant human glutamic acid decarboxylase-65 (GAD65) antigen is used to confirm GAD65 autoantibody (when suspected from immunofluorescent staining pattern).(Griesmann GE, Kryzer TJ, Lennon VA: Autoantibody profiles of myasthenia gravis and Lambert-Eaton myasthenic syndrome. In: Rose NR, Hamilton RG, et al. eds. *Manual of Clinical and Laboratory Immunology*. 6th ed.ASM Press; 2002:1005-1012; 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)

Immunoblot:

All steps are performed at ambient temperature (18-28 degrees C) utilizing the EUROBlot One instrument.

Diluted patient serum (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 serums will bind to the purified recombinant antigen and negative serums will not bind. Strips are washed to remove unbound antibodies and then incubated with antihuman IgG antibodies (alkaline phosphatase-labelled) and incubated for 30 minutes. The strips are again washed to remove unbound antihuman 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)

Cell Binding Assay:

Patient serum is applied to a composite slide containing transfected and nontransfected HEK-293 cells. After incubation and washing, fluorescein-conjugated goat-antihuman IgG is applied to detect the presence of patient IgG binding.(Package insert: IIFT: Neurology Mosaics, Instructions for the indirect immunofluorescence test.

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EUROIMMUN; FA\_112d-1\_A\_UK\_C13, 02/2019)

**Neuromyelitis Optica-IgG Fluorescence-Activated Cell Sorting Assay:**

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).(Unpublished Mayo method)

**Myelin Oligodendrocyte Glycoprotein-IgG1 Fluorescence-Activated Cell Sorting Assay:**

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 myelin oligodendrocyte glycoprotein (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).(Unpublished Mayo method)

**PDF Report**

No

**Day(s) Performed**

ANN1S, DPPIS, GFAIS, GL1IS, PCATR, AGN1S, AMPIS, ANN2S, ANN3S, DPPTS, GFATS, GL1TS, NMDIS, PCAB2, PCABP, GD65S:

Monday through Sunday

MOGFS, NMOFS, MOGTS, NMOTS:

Monday, Tuesday, Thursday

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

Monday through Friday

CS2CS, GABCS, LG1CS, NMDCS, AMPCS:

Monday through Thursday, Sunday

DPPCS, GL1CS:

Wednesday

GFACS:



Monday, Wednesday

**Report Available**

10 to 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**

86341

86255 x11

**LOINC® Information**

Test ID	Test Order Name	Order LOINC Value
PCDES	Peds Autoimmune CNS Eval, S	In Process

Result ID	Test Result Name	Result LOINC Value
61516	NMDA-R Ab CBA, S	93503-1
61519	GABA-B-R Ab CBA, S	93428-1
605131	Peds Autoimmune CNS Interp, S	69048-7
80150	ANNA-1, S	94342-3
81596	GAD65 Ab Assay, S	94345-6
83076	PCA-Tr, S	94352-2
38324	NMO/AQP4 FACS, S	43638-6
64279	LGI1-IgG CBA, S	94287-0
64281	CASPR2-IgG CBA, S	94285-4
65563	MOG FACS, S	90248-6
64930	DPPX Ab IFA, S	82976-2
64928	mGluR1 Ab IFA, S	94347-2

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Result ID	Test Result Name	Result LOINC Value
605155	GFAP IFA, S	94346-4
36349	Reflex Added	77202-0