Overview

Useful For

Investigating new onset cryptogenic epilepsy with incomplete seizure control and duration of less than 2 years using serum specimens

Investigating new onset cryptogenic epilepsy plus 1 or more of the following accompaniments:

-Psychiatric accompaniments (psychosis, hallucinations)

-Movement disorder (myoclonus, tremor, dyskinesias)

-Headache

-Cognitive impairment/encephalopathy

-Autoimmune stigmata (personal history or family history or signs of diabetes mellitus, thyroid disorder, vitiligo, premature graying of hair, myasthenia gravis, rheumatoid arthritis, systemic lupus erythematosus, idiopathic adrenocortical insufficiency), or multiple sclerosis

-History of cancer

-Smoking history (over 20 pack-years) or other cancer risk factors

-Investigating seizures occurring within the context of a subacute multifocal neurological disorder without obvious cause, especially in a patient with a past or family history of cancer

-A rising autoantibody titer in a previously seropositive patient suggests cancer recurrence

Testing Algorithm

If client requests or if indirect immunofluorescence assay (IFA) patterns suggest collapsin response-mediator protein-5-IgG (CRMP-5-IgG), then CRMP-5-IgG Western blot and acetylcholine receptor (AChR) muscle binding are performed at an additional charge.

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

If IFA pattern suggests antiglial nuclear antibody (AGNA)-1, then AGNA-1 immunoblot is performed at an additional charge.

If IFA pattern suggests antineuronal nuclear antibodies (ANNA)-1, then ANNA-1 immunoblot is performed at an additional charge.

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

If IFA pattern suggests Purkinje cytoplasmic antibody (PCA)-1, then PCA-1 immunoblot is performed at an additional charge.

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

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

If AMPA-R antibody CBA is positive, then CRMP-5-IgG Western blot and AChR (muscle) binding antibody are performed at an additional charge.

If contactin-associated protein-like-2 (CASPR2)-receptor antibody CBA is positive, then CRMP-5-IgG Western blot, and AChR (muscle) binding antibody are performed at an additional charge.

If IFA pattern suggests gamma-aminobutyric acid B (GABA-B)-receptor antibody, and GABA-B-receptor antibody is positive, then GABA-B-receptor antibody IF titer assay is performed at an additional charge.

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

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

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

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

For more information, see Autoimmune/Paraneoplastic Epilepsy Evaluation Algorithm-Serum

Special Instructions

- Autoimmune/Paraneoplastic Epilepsy Evaluation Algorithm-Serum

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
AEPSI	Epilepsy, Interpretation, S	No	Yes
AMPCS	AMPA-R Ab CBA, 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
CS2CS	CASPR2-IgG CBA, S	No	Yes
CRMS	CRMP-5-IgG, 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
NMDCS	NMDA-R Ab CBA, S	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
ARBI	ACh Receptor (Muscle)	Yes	No

	Binding Ab		
AGNBS	AGNA-1 Immunoblot, 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
CRMWS	CRMP-5-IgG Western Blot, S	Yes	No
DPPCS	DPPX Ab CBA, S	No	No
DPPTS	DPPX Ab IFA Titer, 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
NMDIS	NMDA-R Ab IF Titer Assay, S	No	No
PC1BS	PCA-1 Immunoblot, S	No	No
PCTBS	PCA-Tr Immunoblot, S	No	No
PCABP	Purkinje Cell Cytoplasmic Ab Type 1	No	No

Method Name

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

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

CRMWS: Western Blot (WB)

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

ARBI, GD65S: Radioimmunoassay (RIA)

NY State Available

Yes

Specimen

Specimen Type

Serum

Ordering Guidance

Multiple neuroimmunology profile tests are available. For testing that is performed with each profile, see Autoimmune Neurology Antibody Matrix.

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 (IVIg) 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.

3. Patient should have no general anesthetic or muscle-relaxant drugs in the previous 24 hours.

Collection Container/Tube:

Preferred: Red top

Acceptable: Serum gel

Submission Container/Tube: Plastic 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.

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject
Gross icterus	Reject

Specimen Minimum Volume

2.5 mL

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information

Antiepileptic drugs (AED) are the mainstay of treatment for epilepsy, but seizures continue in one-third of patients despite appropriate AED therapeutic trials. The etiology of epilepsy often remains unclear. Seizures are a common symptom in autoimmune neurological disorders, including limbic encephalitis and multifocal paraneoplastic disorders. Seizures may be the exclusive manifestation of an autoimmune encephalopathy without evidence of limbic encephalitis.

Autoimmune epilepsy is increasingly recognized in the spectrum of neurological disorders characterized by detection of neural autoantibodies in serum or spinal fluid (CSF) and responsiveness to immunotherapy. The advent of more sensitive and specific serological detection methods is increasingly revealing previously underappreciated autoimmune epilepsies. Neural autoantibodies specific for intracellular and plasma membrane antigens aid the diagnosis of autoimmune epilepsy, but no single antibody is specific for this diagnosis.

Autoantibody specificities currently most informative for autoimmune epilepsies include leucine-rich glioma inactivated protein-1 (LGI1), glutamic acid decarboxylase-65 (GAD65), N-methyl-D-aspartate receptor (NMDA-R), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA-R), and gamma-aminobutyric acid type B receptor (GABA-B-R) antibodies.

Autoantibodies recognizing onconeural proteins shared by neurons, glia, or muscle (eg, antineuronal nuclear antibody, type 1 [ANNA 1]; collapsin response-mediator protein-5 neuronal [CRMP-5-IgG]; N-type calcium channel antibody), also serve as markers of paraneoplastic or idiopathic autoimmune epilepsies. A specific neoplasm is often predictable by the individual patient's autoantibody profile.

Suspicion for autoimmune epilepsy on clinical grounds justifies comprehensive evaluation of CSF and serum for neural autoantibodies. Selective testing for individual autoantibodies is not advised because each is individually rare, and a timely diagnosis is critical. Collectively, the antibodies tested for in the autoimmune epilepsy evaluations represent a broad spectrum of treatable disorders, some of which are associated with occult cancer. Testing of CSF for autoantibodies is particularly helpful when serum testing is negative, though in some circumstances testing both serum and CSF simultaneously is pertinent. Testing of CSF is recommended for some antibodies in particular (such as NMDA-R antibody and glial fibrillary acidic protein [GFAP]-IgG) because CSF testing is both more sensitive and specific. In contrast, serum testing for LGI1 antibody is more sensitive than CSF testing. Failure to detect a neural antibody does not exclude the diagnosis of autoimmune epilepsy when other clinical clues exist. A trial of immunotherapy is justifiable in those cases.

Reference Values

Test ID	Reporting Name	Methodology*	Reference Value
AEPSI	Epilepsy, Interpretation, S	Medical interpretation	NA
AMPCS	AMPA-R Ab CBA, S	CBA	Negative
AMPHS	Amphiphysin Ab, S	IFA	<1:240
AGN1S	Anti-Glial Nuclear Ab, Type 1	IFA	<1:240
ANN1S	Anti-Neuronal Nuclear Ab, Type 1	IFA	<1:240
ANN2S	Anti-Neuronal Nuclear Ab, Type 2	IFA	<1:240
ANN3S	Anti-Neuronal Nuclear Ab, Type 3	IFA	<1:240
CS2CS	CASPR2-IgG CBA, S	CBA	Negative
CRMS	CRMP-5-IgG, S	IFA	<1:240
DPPIS	DPPX Ab IFA, S	IFA	Negative
GABCS	GABA-B-R Ab CBA, S	CBA	Negative
GD65S	GAD65 Ab Assay, S	RIA	< or =0.02 nmol/L Reference values apply to all ages
GFAIS	GFAP IFA, S	IFA	Negative
LG1CS	LGI1-IgG CBA, S	CBA	Negative
GL1IS	mGluR1 Ab IFA, S	IFA	Negative
NMDCS	NMDA-R Ab CBA, S	CBA	Negative
PCAB2	Purkinje Cell Cytoplasmic Ab Type 2	IFA	<1:240
PCATR	Purkinje Cell Cytoplasmic Ab Type Tr	IFA	<1:240

Test ID	Reporting Name	Methodology*	Reference Value
ARBI	ACh Receptor (Muscle) Binding Ab	RIA	< or =0.02 nmol/L
AGNBS	AGNA-1 Immunoblot, S	IB	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
CRMWS	CRMP-5-IgG Western Blot, S	WB	Negative
DPPCS	DPPX Ab CBA, S	CBA	Negative
DPPTS	DPPX Ab IFA Titer, S	IFA	<1:240
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
NMDIS	NMDA-R Ab IF Titer Assay, S	IFA	<1:120
PC1BS	PCA-1 Immunoblot, S	IB	Negative
PCTBS	PCA-Tr Immunoblot, S	IB	Negative
PCABP	Purkinje Cell Cytoplasmic Ab Type 1	IFA	<1:240

*Methodology abbreviations:

Immunofluorescence assay (IFA)

Cell-binding assay (CBA)

Western blot (WB)

Radioimmunoassay (RIA)

Immunoblot (IB)

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."

Note: CRMP-5 titers lower than 1:240 are detectable by recombinant CRMP-5 Western blot analysis. CRMP-5 Western blot analysis will be done on request on stored serum (held 4 weeks). This supplemental testing is recommended in cases of chorea, vision loss, cranial neuropathy, and myelopathy. Call the Neuroimmunology Laboratory at 800-533-1710 to request CRMP-5 Western blot.

Interpretation

Antibodies specific for neuronal, glial, or muscle proteins are valuable serological markers of autoimmune epilepsy and of a patient's immune response to cancer. These autoantibodies are not found in healthy subjects and are usually accompanied by subacute neurological symptoms and signs. It is not uncommon for more than 1 of the following autoantibodies to be detected in patients with autoimmune dementia.

-Plasma membrane antibodies (N-methyl-D-aspartate [NMDA] receptor; 2-amino-3-[5-methyl-3-oxo-1,2-oxazol-4-yl] propanoic acid [AMPA] receptor; gamma-amino butyric acid [GABA-B] receptor). These autoantibodies are all potential effectors of dysfunction.

-Antineuronal nuclear antibody, type 1 (ANNA-1) or type 3 (ANNA-3).

-Neuronal or muscle cytoplasmic antibodies (amphiphysin, Purkinje cell antibody-type 2 [PCA-2], collapsin response-mediator protein-5 neuronal [CRMP-5-IgG], or glutamic acid decarboxylase [GAD65] antibody).

Cautions

Negative results do not exclude autoimmune epilepsy or cancer.

This test does not detect Ma2 antibody (alias MaTa). Ma2 antibody has been described in patients with brainstem and limbic encephalitis in the context of testicular germ cell neoplasms. Scrotal ultrasound is advisable in men who present with unexplained subacute encephalitis.

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

Clinical Reference

1. Quek AM, Britton JW, McKeon A, et al: Autoimmune epilepsy: clinical characteristics and response to immunotherapy. Arch Neurol. 2012 May;69(5):582-593

2. Yu Z, Kryzer TJ, Griesmann GE, Kim K, Benarroch EE, Lennon VA: CRMP-5 neuronal autoantibody: marker of lung cancer and thymoma-related autoimmunity. Ann Neurol. 2001 Feb;49(2):146-154

3. Pittock SJ, Yoshikawa H, Ahlskog JE, et al: Glutamic acid decarboxylase autoimmunity with brainstem, extrapyramidal and spinal cord dysfunction. Mayo Clin Proc. 2006 Sep;81:1207-1214

4. Klein CJ, Lennon VA, Aston PA, et al: Insights from LGI1 and CASPR2 potassium channel complex autoantibody subtyping. JAMA Neurol. 2013 Feb;70(2):229-234

5. Lancaster E, Martinez-Hernandez E, Dalmau J: Encephalitis and antibodies to synaptic and neuronal cell surface proteins. Neurology. 2011 Jul;77(2):179-189

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 Nov;56(5):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 (125)I-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: Rose NR, Hamilton RG, 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 type 1 diabetes mellitus. Mayo Clin Proc. 1998 Dec;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, Kim K, Benarroch EE, Lennon VA: CRMP-5 neuronal autoantibody: marker of lung cancer and thymoma-related autoimmunity. Ann Neurol. 2001 Feb;49[2]:146-154; Dubey D, Jitprapaikulsan J, Bi H, et al: Amphiphysin-IgG autoimmune neuropathy: A recognizable clinicopathologic syndrome. Neurology. 2019 Nov 12;93[20]:e1873-e1880)

Immunoblot:

All steps are performed at ambient temperature (18-28 degrees C) utilizing the EUROBlot One instrument. Diluted patient specimen (1:101) 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 serum antibodies and then incubated with anti-human IgG antibodies (alkaline phosphatase-labelled) 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 specimen 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. EUROIMMUN; FA_112d-1_A_UK_C13, 02/25/2019)

PDF Report
No

Specimen Retention Time
28 days

Performing Laboratory Location
Rochester

Fees & Codes

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 US Food and Drug Administration.

CPT Code Information
86255 x 16

86341

83519-ARBI (if appropriate)

84182-AGNBS (if appropriate)

86256-AMPIS (if appropriate)

84182-AMIBS (if appropriate)

84182-AN1BS (if appropriate)

84182-AN2BS (if appropriate)

84182-CRMWS (if appropriate)

86255-DPPCS (if appropriate)

86256-DPPTS (if appropriate)

86256-GABIS (if appropriate)

86255-GFACS (if appropriate)

86256-GFATS (if appropriate)

86255-GL1CS (if appropriate)

86256-GL1TS (if appropriate)

86256-NMDIS (if appropriate)

84182-PC1BS (if appropriate)

84182-PCTBS (if appropriate)

86255-PCABP (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
EPS2	Epilepsy, Autoimm/Paraneo, S	94698-8

Result ID	Reporting Name	LOINC®
89080	AGNA-1, S	94341-5
81722	Amphiphysin Ab, S	94340-7
80150	ANNA-1, S	94342-3

36349	Reflex Added	77202-0
80776	ANNA-2, S	94343-1
83137	ANNA-3, S	94344-9
83077	CRMP-5-IgG, S	94815-8
81596	GAD65 Ab Assay, S	94345-6
83138	PCA-2, S	94351-4
83076	PCA-Tr, S	94352-2
61516	NMDA-R Ab CBA, S	93503-1
61518	AMPA-R Ab CBA, S	93489-3
61519	GABA-B-R Ab CBA, S	93428-1
34259	Epilepsy, Interpretation, S	69048-7
64279	LGI1-IgG CBA, S	94287-0
64281	CASPR2-IgG CBA, S	94285-4
64930	DPPX Ab IFA, S	82976-2
64928	mGluR1 Ab IFA, S	94347-2
605155	GFAP IFA, S	94346-4