

Overview

Useful For

Aids in the diagnosis of paraneoplastic neurological autoimmune disorders related to carcinoma of lung, breast, ovary, thymoma, or Hodgkin lymphoma in spinal fluid specimens

Testing Algorithm

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

If IFA pattern suggests antineuronal nuclear antibodies (ANNA)-1 antibody, 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 antibody, 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 patterns suggest collapsin response-mediator protein (CRMP)-5-IgG, then CRMP-5-IgG Western blot is performed at an additional charge.

If IFA patterns suggest glutamic acid decarboxylase 65 (GAD65) antibody, then GAD65 antibody radioimmunoassay (RIA) is performed at an additional charge.

If IFA patterns suggest neuronal voltage-gated potassium channel-complex (VGKC) autoantibody, then VGKC-complex antibody RIA is performed at an additional charge.

If VGKC-complex antibody RIA result is greater than 0.00 nmol/L, then leucine-rich, glioma inactivated 1 (LGI1)-IgG cell-binding assay (CBA) and contactin-associated protein-like 2 (CASPR2)-IgG CBA 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 N-methyl-D-aspartate (NMDA)-receptor, then NMDA-receptor antibody CBA and/or NMDA-receptor antibody IFA titer is performed at an additional charge.

If IFA pattern suggests alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-receptor, then AMPA-Receptor antibody CBA and/or AMPA-receptor antibody IFA titer is performed at an additional charge.

If IFA pattern suggests gamma-aminobutyric acid B (GABA-B)-receptor, then GABA-B-receptor antibody CBA and/or GABA-B-Receptor antibody IFA titer is performed at an additional charge.

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

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

For more information. the following algorithms are available:

[-Paraneoplastic Evaluation Algorithm-Spinal Fluid](#)

[-Hereditary Peripheral Neuropathy Diagnostic Algorithm](#)

Special Instructions

- [Paraneoplastic Evaluation Algorithm-Spinal Fluid](#)
- [Hereditary Peripheral Neuropathy Diagnostic Algorithm](#)

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
PNEOI	Paraneoplastic Interpretation, 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
CRMC	CRMP-5-IgG, CSF	No	Yes
PCTRC	Purkinje Cell Cytoplasmic 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
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
CS2CC	CASPR2-IgG CBA, CSF	No	No
CRMWC	CRMP-5-IgG Western Blot, CSF	Yes	No
DPPCC	DPPX Ab CBA, CSF	No	No
DPPTC	DPPX Ab IFA Titer, CSF	No	No
DPPIC	DPPX Ab IFA, CSF	No	No
GABCC	GABA-B-R Ab CBA, CSF	No	No
GABIC	GABA-B-R Ab IF Titer Assay, CSF	No	No
GD65C	GAD65 Ab Assay, CSF	Yes	No
LG1CC	LGI1-IgG CBA, CSF	No	No
GL1CC	mGluR1 Ab CBA, CSF	No	No
GL1TC	mGluR1 Ab IFA Titer, CSF	No	No
GL1IC	mGluR1 Ab IFA, CSF	No	No

NMDCC	NMDA-R Ab CBA, CSF	No	No
NMDIC	NMDA-R Ab IF Titer Assay, CSF	No	No
PC1BC	PCA-1 Immunoblot, CSF	No	No
PCTBC	PCA-Tr Immunoblot, CSF	No	No
VGKCC	VGKC-complex Ab IPA, CSF	No	No

Method Name

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

CRMWC: Western Blot

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

GD65C, VGKCC: Radioimmunoassay (RIA)

AMPCC, CS2CC, DPPCC, GABCC, GL1CC, LG1CC, NMDCC: Cell-Binding Assay (CBA)

NY State Available

Yes

Specimen

Specimen Type

CSF

Additional Testing Requirements

In patients with a history of tobacco use or other lung cancer risk, or if thymoma is suspected, PAVAL / Paraneoplastic Autoantibody Evaluation, Serum is also recommended.

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

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 mL

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information

Several antineuronal and glial autoantibodies are recognized clinically as markers of a patient's immune response to specific cancers (paraneoplastic autoantibodies). Seropositive patients present with neurologic symptoms and signs in more than 90% of cases. The cancers are most commonly small-cell lung carcinoma, ovarian (or related mullerian) carcinoma, breast carcinoma, thymoma, or Hodgkin lymphoma. The cancers may be new or recurrent, are usually limited in metastatic volume, and are often occult by standard imaging procedures. Detection of the informative marker autoantibodies allows early diagnosis and treatment of the cancer, which may lessen neurological morbidity and improve survival.

Serum is the preferred specimen for paraneoplastic autoantibodies. However, cerebrospinal fluid (CSF) results are sometimes positive when serum results are negative (especially for collapsin response-mediator protein-5-IgG [CRMP-5] and other inflammatory central nervous system autoimmunity). Additionally, CSF is more readily interpretable because it generally lacks the interfering nonorgan-specific antibodies that are common in the serum of patients with cancer.

Because neurologists typically perform spinal taps in these patients, the recommendation is to submit CSF specimens with serum specimens, either for simultaneous testing or to be held for testing only if serum is negative.

CRMP-5-IgG Western blot is also performed by specific request for more sensitive detection of CRMP-5-IgG. Testing should be requested in cases of subacute basal ganglionic disorders (chorea, Parkinsonism), cranial neuropathies (especially loss of vision, taste, or smell), and myelopathies.

Reference Values

Test ID	Reporting name	Methodology	Reference value
AMPHC	Amphiphysin Ab, CSF	Indirect immunofluorescence (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
CRMC	CRMP-5-IgG, CSF	IFA	<1:2
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
AMPCC	AMPA-R Ab CBA, CSF	Cell-binding assay (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
CRMWC	CRMP-5-IgG Western Blot, CSF	Western blot (WB)	Negative
CS2CC	CASPR2-IgG CBA, CSF	CBA	Negative
DPPCC	DPPX Ab CBA, CSF	CBA	Negative
DPPIC	DPPX Ab IFA, CSF	IFA	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
GD65C	GAD65 Ab Assay, CSF	Radioimmunoassay (RIA)	< or =0.02 nmol/L
LG1CC	LGI1-IgG CBA, CSF	CBA	Negative
GL1CC	mGluR1 Ab CBA, CSF	CBA	Negative
GL1IC	mGluR1 Ab IFA, CSF	IFA	Negative

GL1TC	mGluR1 Ab IFA Titer, CSF	IFA	<1:2
NMDCC	NMDA-R Ab CBA, CSF	CBA	Negative
NMDIC	NMDA-R Ab IF Titer Assay, CSF	IFA	<1:2
PC1BC	PCA-1 Immunoblot, CSF	IB	Negative
PCTBC	PCA-Tr Immunoblot, CSF	IB	Negative
VGKCC	VGKC-complex Ab IPA, CSF	RIA	< or =0.02 nmol/L

Neuron-restricted patterns of IgG staining that do not fulfill criteria for amphiphysin, ANNA-1, ANNA-2, ANNA-3, AGNA-1, PCA-1, PCA-2, PCA-Tr, or CRMP-5-IgG may be reported as "unclassified antineuronal IgG." Complex patterns that include non-neuronal elements may be reported as "uninterpretable."

Note: Titers lower than 1:2 are detectable by recombinant CRMP-5 Western blot analysis. CRMP-5 Western blot analysis will be done on request on stored spinal fluid (held 4 weeks). This supplemental testing is recommended in cases of chorea, vision loss, cranial neuropathy, and myelopathy. Call 800-533-1710 to request CRMP-5 Western blot.

Interpretation

Antibodies directed at onconeural proteins shared by neurons, glia, muscle, and certain cancers are valuable serological markers of a patient's immune response to cancer. They are not found in healthy subjects, and are usually accompanied by subacute neurological symptoms and signs. Several autoantibodies have a syndromic association, but no autoantibody predicts a specific neurological syndrome. Conversely, a positive autoantibody profile has 80% to 90% predictive value for a specific cancer. It is not uncommon for more than one paraneoplastic autoantibody to be detected, each predictive of the same cancer.

Cautions

No significant cautionary statements

Clinical Reference

1. Lucchinetti CF, Kimmel DW, Lennon VA: Paraneoplastic and oncological profiles of patients seropositive for type 1 anti-neuronal nuclear antibodies. *Neurology*. 1998 Mar;50(3):652-657
2. Graus F, Vincent A, Pozo-Rosich P, et al: Anti-glial nuclear antibody: marker of lung cancer-related paraneoplastic neurological syndromes. *J Neuroimmunol*. 2005 Aug;165(1-2):166-171
3. Pittock SJ, Lucchinetti CF, Lennon VA: Anti-neuronal nuclear autoantibody type 2: paraneoplastic accompaniments. *Ann Neurol*. 2003 May;53(5):580-587
4. Chan KH, Vernino S, Lennon VA: ANNA-3 anti-neuronal nuclear antibody: marker of lung cancer-related autoimmunity. *Ann Neurol*. 2001 Sep;50(3):301-311
5. Hetzel DJ, Stanhope CR, O'Neill BP, Lennon VA: Gynecologic cancer in patients with subacute cerebellar degeneration predicted by anti-Purkinje cell antibodies and limited in metastatic volume. *Mayo Clin Proc*. 1990 Dec;65(12):1558-1563

6. Pittock SJ, Lucchinetti CF, Parisi JE, et al: Amphiphysin autoimmunity: paraneoplastic accompaniments. *Ann Neurol*. 2005 Jul;58(1):96-107

7. McKeon A, Pittock SJ: Paraneoplastic encephalomyelopathies: pathology and mechanisms. *Acta Neuropathol*. 2011 Oct;122(4):381-400

8. Horta ES, Lennon VA, Lachance DH, et al: Neural autoantibody clusters aid diagnosis of cancer. *Clin Cancer Res*. 2014 Jul 15;20(14):3862-3869

Performance

Method Description

Indirect Immunofluorescence Assay (IFA):

The patient's sample is tested by a standardized IFA that uses a composite frozen section of mouse cerebellum, kidney, and gut tissues. After incubation with sample and washing, fluorescein-conjugated goat-antihuman IgG is applied. Neuron-specific autoantibodies are identified by their characteristic fluorescence staining patterns. Samples that are scored positive for any neuronal nuclear or cytoplasmic autoantibody are titrated to an endpoint.

Interference by coexisting non-neuron-specific autoantibodies can usually be eliminated by serologic absorption.(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.0000000000000385689)

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 (IB):

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 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)

Radioimmunoassay (RIA):

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.(Vernino S, Kryzer TJ, Lennon AV: Autoimmune autonomic neuropathy and neuromuscular hyperexcitability disorders. In: Rose NR, Hamilton RG, Detrick B, eds. *Manual of Clinical and Laboratory Immunology*. 6th ed. ASM Press; 2002:1013-1017; 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)

Cell-Binding Assay (CBA):

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. EUROIMMUN; FA_112d-1_A_UK_C13, 02/2019)

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 9

84182-AGNBC (if appropriate)

86255-AMPCC (if appropriate)

86256-AMPIC (if appropriate)

84182-AMIBC (if appropriate)

84182-AN1BC (if appropriate)

84182-AN2BC (if appropriate)

86255-CS2CC (if appropriate)

84182-CRMWC (if appropriate)

86255-DPPCC (if appropriate)

86256-DPPTC (if appropriate)

86255-DPPIC (if appropriate)

86255-GABCC (if appropriate)

86256-GABIC (if appropriate)

86341-GD65C (if appropriate)

86255-LG1CC (if appropriate)

86255-GL1CC (if appropriate)

86256-GL1TC (if appropriate)

86255-GL1IC (if appropriate)

86255-NMDCC (if appropriate)

86256-NMDIC (if appropriate)

84182-PC1BC (if appropriate)

84182-PCTBC (if appropriate)

83519-VGKCC (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
PAC1	Paraneoplas Autoantibody Eval,CSF	94818-2

Result ID	Reporting Name	LOINC®
89079	AGNA-1, CSF	94355-5
5906	Amphiphysin Ab, CSF	94354-8
3852	ANNA-1, CSF	94356-3
36429	Reflex Added	77202-0
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
34271	Paraneoplastic Interpretation, CSF	69048-7