

## Overview

### Useful For

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

### 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
AMIBC	Amphiphysin Immunoblot, CSF	No	No
AN1BC	ANNA-1 Immunoblot, CSF	No	No
AN2BC	ANNA-2 Immunoblot, CSF	No	No
CRMWC	CRMP-5-IgG Western Blot, CSF	Yes	No
PC1BC	PCA-1 Immunoblot, CSF	No	No
PCTBC	PCA-Tr Immunoblot, CSF	No	No
AGNTC	AGNA-1 Titer, CSF	No	No

AN1TC	ANNA-1 Titer, CSF	No	No
AN2TC	ANNA-2 Titer, CSF	No	No
AN3TC	ANNA-3 Titer, CSF	No	No
APHTC	Amphiphysin Ab Titer, CSF	No	No
CRMTC	CRMP-5-IgG Titer, CSF	No	No
PC1TC	PCA-1 Titer, CSF	No	No
PC2TC	PCA-2 Titer, CSF	No	No
PCTTC	PCA-Tr Titer, CSF	No	No

### Testing Algorithm

If the indirect immunofluorescence assay (IFA) pattern suggests AGNA-1 antibody, then AGNA-1 immunoblot (IB) and AGNA-1 IFA titer will be performed at an additional charge.

If the IFA pattern suggests antineuronal nuclear antibody type 1 (ANNA-1), then ANNA-1 IB, ANNA-1 IFA titer, and ANNA-2 IB will be performed at an additional charge.

If the IFA pattern suggests ANNA-2 antibody, then ANNA-2 IB, ANNA-2 IFA titer, and ANNA-1 IB will be performed at an additional charge.

If the IFA pattern suggests ANNA-3 antibody, then ANNA-3 titer will be performed at an additional charge.

If the IFA pattern suggests Purkinje cytoplasmic antibody type 1 (PCA-1), then PCA-1 IB, and PCA-1 IFA titer will be performed at an additional charge.

If the IFA pattern suggests PCA-2 antibody, then PCA-2 IFA titer will be performed at an additional charge.

If the IFA pattern suggests PCA-Tr antibody, then PCA-Tr immunoblot, and PCA-Tr IFA titer will be performed at an additional charge.

If client requests or the IFA patterns suggest CRMP-5-IgG, then CRMP-5-IgG IFA titer and CRMP-5-IgG western blot will be performed at an additional charge.

If the IFA patterns suggest amphiphysin antibody, then amphiphysin IB and amphiphysin IFA titer will be performed at an additional charge.

The following algorithm is available: [Paraneoplastic Evaluation Algorithm-Spinal Fluid](#).

### Special Instructions

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

### Method Name

AGN1C, AMPHC, ANN1C, ANN2C, ANN3C, CRMC, PCA1C, PCA2C7, PCTRC, AGNTC, APHTC, AN1TC, AN2TC, AN3TC, CRMTC, PC1TC, PC2TC, PCTTC: Indirect Immunofluorescence Assay (IFA)

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

**NY State Available**

Yes

**Specimen****Specimen Type**

CSF

**Ordering Guidance**

This test **no longer contains all known, clinically relevant antibodies** for patients suspected of autoimmune neurological disorders. Instead, consider a comprehensive neurological phenotype-specific autoimmune/paraneoplastic evaluation (eg, encephalopathy, movement disorders, myopathy, axonal neuropathy). For more information as well as phenotype-specific testing options, refer to [Autoimmune Neurology Test Ordering Guide](#) or the [Neurology specialty website](#).

**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

**Preferred:** Vial number 2

**Acceptable:** Any vial

**Specimen Volume:** 4 mL

**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	
	Ambient	72 hours	
	Frozen	28 days	

**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 signs and symptoms 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	IFA	Negative
AGN1C	Anti-Glial Nuclear Ab, Type 1	IFA	Negative
ANN1C	Anti-Neuronal Nuclear Ab, Type 1	IFA	Negative
ANN2C	Anti-Neuronal Nuclear Ab, Type 2	IFA	Negative
ANN3C	Anti-Neuronal Nuclear Ab, Type 3	IFA	Negative
CRMC	CRMP-5-IgG, CSF	IFA	Negative
PCTRC	Purkinje Cell Cytoplasmic Ab Type Tr	IFA	Negative
PCA1C	Purkinje Cell Cytoplasmic Ab Type 1	IFA	Negative
PCA2C	Purkinje Cell Cytoplasmic Ab Type 2	IFA	Negative

**Reflex Information:**

Test ID	Reporting name	Methodology	Reference value
AGNBC	AGNA-1 Immunoblot, CSF	IB	Negative
AGNTC	AGNA-1 Titer, CSF	IFA	<1:2
AMIBC	Amphiphysin Immunoblot, CSF	IB	Negative
AN1BC	ANNA-1 Immunoblot, CSF	IB	Negative
AN1TC	ANNA-1 Titer, CSF	IFA	<1:2
AN2BC	ANNA-2 Immunoblot, CSF	IB	Negative
AN2TC	ANNA-2 Titer, CSF	IFA	<1:2
AN3TC	ANNA-3 Titer, CSF	IFA	<1:2
APHTC	Amphiphysin Ab Titer, CSF	IFA	<1:2
CRMTC	CRMP-5-IgG Titer, CSF	IFA	<1:2
CRMWC	CRMP-5-IgG Western Blot, CSF	WB	Negative
PC1BC	PCA-1 Immunoblot, CSF	IB	Negative
PC1TC	PCA-1 Titer, CSF	IFA	<1:2
PC2TC	PCA-2 Titer, CSF	IFA	<1:2
PCTBC	PCA-Tr Immunoblot, CSF	IB	Negative
PCTTC	PCA-Tr Titer, CSF	IFA	<1:2

\*Methodology abbreviations:

Immunofluorescence assay (IFA)

Western blot (WB)

Immunoblot (IB)

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 signs and symptoms. 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 antibody. *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 encephalomyopathies: 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 specimen is tested by a standardized immunofluorescence assay that uses a composite frozen section of mouse cerebellum, kidney, and gut tissues. After incubation with the specimen and washing, fluorescein-conjugated goat-antihuman IgG is applied. Neuron-specific autoantibodies are identified by their characteristic fluorescence staining patterns. Specimens that are scored positive for any neuronal nuclear or cytoplasmic autoantibody are titrated.

Interference by coexisting non-neuron-specific autoantibodies can usually be eliminated by serologic absorption.(Honarat JA, Komorowski L, Josephs KA, et al. IgLON5 antibody: Neurological accompaniments and outcomes in 20 patients. *Neurol Neuroimmunol Neuroinflamm*. 2017;4[5]:e385. Published 2017 Jul 18.

doi:10.1212/NXI.0000000000000385)

#### 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 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 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.0000000000000552)

**PDF Report**

No

**Day(s) Performed**

Profile tests: Monday through Sunday; Reflex tests: Varies

**Report Available**

10 to 17 days

**Specimen Retention Time**

28 days

**Performing Laboratory Location**

Mayo Clinic Laboratories - Rochester Main Campus

**Fees & 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 account representative. 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. It has not been cleared or approved by the US Food and Drug Administration.

**CPT Code Information**

86255 x 9

84182-AGNBC (if appropriate)

86256-AGNTC (if appropriate)

84182-AMIBC (if appropriate)

84182-AN1BC (if appropriate)

86256-AN1TC (if appropriate)

84182-AN2BC (if appropriate)  
86256-AN2TC (if appropriate)  
86256-AN3TC (if appropriate)  
86256-APHTC (if appropriate)  
86256-CRMTC (if appropriate)  
84182-CRMWC (if appropriate)  
84182-PC1BC (if appropriate)  
86256-PC1TC (if appropriate)  
86256-PC2TC (if appropriate)  
84182-PCTBC (if appropriate)  
86256-PCTTC (if appropriate)

**LOINC® Information**

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

Result ID	Test Result Name	Result LOINC® Value
89079	AGNA-1, CSF	90827-7
5906	Amphiphysin Ab, CSF	90815-2
3852	ANNA-1, CSF	44768-0
7472	ANNA-2, CSF	56959-0
21633	ANNA-3, CSF	90836-8
21650	CRMP-5-IgG, CSF	63216-6
3988	PCA-1, CSF	90841-8
21632	PCA-2, CSF	90843-4
21631	PCA-Tr, CSF	90845-9
34271	Paraneoplastic Interpretation, CSF	57771-8
619519	IFA Notes	48767-8