MAYO CLINIC LABORATORIES

Test Definition: PAVAL

Paraneoplastic, Autoantibody Evaluation, Serum

Overview

Useful For

Serological evaluation of patients who present with a subacute neurological disorder of undetermined etiology, especially those with known risk factors for cancer

Directing a focused search for cancer

Investigating neurological symptoms that appear during, or after, cancer therapy and are not explainable by metastasis

Differentiating autoimmune neuropathies from neurotoxic effects of chemotherapy

Monitoring the immune response of seropositive patients during cancer therapy

Detecting early evidence of cancer recurrence in previously seropositive patients

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
PAINT	Interpretive Comments	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
CRMS	CRMP-5-IgG, S	No	Yes
VGKC	Neuronal (V-G) K+ Channel Ab, S	No	Yes
CCPQ	P/Q-Type Calcium Channel Ab	No	Yes
PCABP	Purkinje Cell Cytoplasmic Ab Type 1	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
AGNBS	AGNA-1 Immunoblot, S	No	No
AMIBS	Amphiphysin Immunoblot, S	No	No
AN1BS	ANNA-1 Immunoblot, S	No	No
AN2BS	ANNA-2 Immunoblot, S	No	No
CS2CS	CASPR2-IgG CBA, S	No	No
CRMWS	CRMP-5-IgG Western Blot, S	Yes	No
LG1CS	LGI1-IgG CBA, S	No	No
PC1BS	PCA-1 Immunoblot, S	No	No
PCTBS	PCA-Tr Immunoblot, S	No	No
AGNTS	AGNA-1 Titer, S	No	No
APHTS	Amphiphysin Ab Titer, S	No	No
AN1TS	ANNA-1 Titer, S	No	No
AN3TS	ANNA-3 Titer, S	No	No
CRMTS	CRMP-5-IgG Titer, S	No	No
PC1TS	PCA-1 Titer, S	No	No
PC2TS	PCA-2 Titer, S	No	No
PCTTS	PCA-Tr Titer, S	No	No
AN2TS	ANNA-2 Titer, S	No	No

Testing Algorithm

If the immunofluorescence assay (IFA) patterns suggest antiglial nuclear antibody-1 (AGNA-1) antibody, then AGNA-1 immunoblot and AGNA-1 IFA titer will be performed at an additional charge.

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

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

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

If voltage-gated potassium channel (VGKC) is above 0.00 nmol/L, then leucine-rich, glioma inactivated 1 (LGI1)-IgG cell-binding assay (CBA) and contactin-associated protein-like 2 (CASPR2)-IgG will be performed at an additional charge.

If the collapsin response-mediator protein (CRMP) IFA is positive, then CRMP-5-IgG Western blot and CRMP-5-IgG IFA titer will be performed at an additional charge.

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.

The following algorithms are available:
-Paraneoplastic Evaluation Algorithm-Serum
-Hereditary Peripheral Neuropathy Diagnostic Algorithm

Special Instructions

- Paraneoplastic Evaluation Algorithm-Serum

Method Name

PAINT: Medical Interpretation

AGN1S, AGNTS, AMPHS, APHTS, ANN1S, ANN2S, ANN3S, AN2TS, AN3TS, CRMS, CRMTS, PCABP, PC1TS, PCAB2, PC2TS, PCATR, PCTTS, AN1TS: Indirect Immunofluorescence Assay (IFA)

CCPQ, VGKC: Radioimmunoassay (RIA)

CRMWS: Western Blot (WB)

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

CS2CS, LG1CS: Cell-Binding Assay (CBA)

NY State Available

Yes

Specimen

Specimen Type

Serum

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, myelopathy, axonal neuropathy). For more information as well as phenotype-specific testing options, refer to Autoimmune Neurology Test Ordering Guide or the Neurology specialty website.

This test **should not be requested** for 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.

Necessary Information

Provide the following information:
-Relevant clinical information
-Ordering provider name, phone number, mailing address, and e-mail address

Specimen Required

Patient Preparation: For optimal antibody detection, specimen collection is recommended prior to initiation of immunosuppressant medication or intravenous immunoglobulin treatment.
Supplies: Sarstedt Aliquot Tube, 5 mL (T914)
Collection Container/Tube:
Preferred: Red top
Acceptable: Serum gel
Submission Container/Tube: Plastic vial
Specimen Volume: 4 mL
Collection Instructions: Centrifuge and aliquot serum into a plastic vial.

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 & Interpretive

Clinical Information

Paraneoplastic autoimmune neurological disorders reflect a patient's humoral and cellular immune responses to cancer. The cancer may be new or recurrent, is usually limited in metastatic volume, and is often occult by standard imaging procedures. Autoantibodies specific for onconeural proteins found in the plasma membrane, cytoplasm, and nucleus of neurons, glia, or muscle are generated in this immune response and serve as serological markers of paraneoplastic autoimmunity. Cancers recognized in this context most commonly are small-cell lung carcinoma, thymoma, ovarian (or related Mullerian) carcinoma, breast carcinoma, and Hodgkin lymphoma. Pertinent childhood neoplasms recognized thus far include neuroblastoma, thymoma, Hodgkin lymphoma, and chondroblastoma. An individual patient's autoantibody profile can predict a specific neoplasm with 90% certainty but not the neurological syndrome.

Four classes of autoantibodies are recognized in this evaluation:
-Antineuronal nuclear antibodies (ANNA-1, ANNA-2, ANNA-3)
-Anti-glial/neuronal nuclear antibodies (AGNA-1; also known as Sox1)
-Neuronal and muscle cytoplasmic antibodies (Purkinje cytoplasmic antibody [PCA]-1, PCA-2, PCA-Tr, collapsin response-mediator protein [CRMP]-5, and amphiphysin)
-Plasma membrane cation channel, P/Q-type calcium channel, and dendrotoxin-sensitive potassium channels. These autoantibodies are potential effectors of neurological dysfunction.

Patients who are seropositive usually present with subacute neurological signs and symptoms, such as encephalopathy, cerebellar ataxia, myelopathy, radiculopathy, plexopathy, or sensory, sensorimotor, or autoimmune neuropathy, with or without a neuromuscular transmission disorder: Lambert-Eaton syndrome, myasthenia gravis, or neuromuscular hyperexcitability. Initial signs may be subtle, but a subacute multifocal and progressive syndrome usually evolves. Sensorimotor neuropathy and cerebellar ataxia are common presentations, but the clinical picture in some patients is dominated by striking gastrointestinal dysmotility, limbic encephalopathy, basal ganglionitis, or cranial neuropathy (especially loss of vision, hearing, smell, or taste).

Cancer risk factors include previous or family history of cancer, history of smoking, or social or environmental exposure to carcinogens. Early diagnosis and treatment of the neoplasm favor less neurological morbidity and offer the best hope for survival.

Reference Values

Test ID	Reporting name	Methodology*	Reference value
PAINT	Interpretive Comments	Medical interpretation	Not applicable
AMPHS	Amphiphysin Ab, S	IFA	Negative
AGN1S	Anti-Glial Nuclear Ab, Type 1	IFA	Negative
ANN1S	Anti-Neuronal Nuclear Ab, Type 1	IFA	Negative

ANN2S	Anti-Neuronal Nuclear Ab, Type 2	IFA	Negative
ANN3S	Anti-Neuronal Nuclear Ab, Type 3	IFA	Negative
CRMS	CRMP-5-IgG, S	IFA	Negative
VGKC	Neuronal (V-G) K+ Channel Ab, S	RIA	< or =0.02 nmol/L
CCPQ	P/Q-Type Calcium Channel Ab	RIA	< or =0.02 nmol/L
PCABP	Purkinje Cell Cytoplasmic Ab Type 1	IFA	Negative
PCAB2	Purkinje Cell Cytoplasmic Ab Type 2	IFA	Negative
PCATR	Purkinje Cell Cytoplasmic Ab Type Tr	IFA	Negative

Reflex Tests:

Test ID	Reporting name	Methodology*	Reference value
AGNBS	AGNA-1 Immunoblot, S	IB	Negative
AGNTS	AGNA-1 Titer, S	IFA	<1:240
AMIBS	Amphiphysin Immunoblot, S	IB	Negative
AN1BS	ANNA-1 Immunoblot, S	IB	Negative
AN1TS	ANNA-1 Titer, S	IFA	<1:240
AN2BS	ANNA-2 Immunoblot, S	IB	Negative
AN2TS	ANNA-2 Titer, S	IFA	<1:240
AN3TS	ANNA-3 Titer, S	IFA	<1:240
APHTS	Amphiphysin Ab Titer, S	IFA	<1:240
CRMTS	CRMP-5-IgG Titer, S	IFA	<1:240
CS2CS	CASPR2-IgG CBA, S	CBA	Negative
CRMWS	CRMP-5-IgG Western Blot, S	WB	Negative
LG1CS	LGI1-IgG CBA, S	CBA	Negative
PC1BS	PCA-1 Immunoblot, S	IB	Negative
PC1TS	PCA-1 Titer, S	IFA	<1:240
PC2TS	PCA-2 Titer, S	IFA	<1:240
PCTBS	PCA-Tr Immunoblot, S	IB	Negative
PCTTS	PCA-Tr Titer, S	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 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: 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 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

Negative results do not exclude cancer.

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

This evaluation does not include Ma2 autoantibody (also known as MaTa). Ma2 autoantibody 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. N-methyl-D-aspartate receptor antibodies have been reported in women with paraneoplastic encephalitis related to ovarian teratoma.

Clinical Reference

1. McKeon A, Pittock SJ: Paraneoplastic encephalomyelopathies: pathology and mechanisms. Acta Neuropathol. 2011 Oct;122(4):381-400
2. Horta ES, Lennon VA, Lachance DH, et al: Neural autoantibody clusters aid diagnosis of cancer. Clin Cancer Res. 2014 Jun;20(14):3862-3869

Performance

Method Description

Indirect Immunofluorescence Assay:
The patient's specimen is tested by a standardized indirect immunofluorescence assay (IFA) that uses a composite frozen section of mouse cerebellum, kidney, and gut tissues. After incubation with specimen 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.0000000000000385

Radioimmunoassay:
(125)I-labeled recombinant human antigens or labeled receptors are incubated with patient sample. After incubation, anti-human IgG is added to form an immunoprecipitate. The amount of (125)I-labeled antigen in the immunoprecipitate is measured using a gamma-counter. The amount of gamma emission in the precipitate is proportional to the amount of

antigen-specific IgG in the sample. Results are reported as units of precipitated antigen (nMol) per L of patient sample.(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;72[11]:1304-1312 doi:10.1001/jamaneurol.2015.2378)

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

Immunoblot:
All steps are performed at room temperature (18-28 degrees C) utilizing the EUROBlot One instrument. Diluted patient serum (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 serums will bind to the purified recombinant antigen, and negative serums will not bind. Strips are washed to remove unbound serum antibodies and then incubated with anti-human IgG antibodies (alkaline phosphatase-labelled) and incubated for 30 minutes. The strips are again washed to remove unbound anti-human IgG antibodies, and nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate 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;6[3]:e552. doi:10.1212/NXI.0000000000000552)

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;93(20):e1873-e1880. doi:10.1212/WNL.0000000000008472)

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

Rochester

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

83519
86596
86255 x 9
84182-AGNBS (if appropriate)
86256-AGNTS (if appropriate)
84182-AMIBS (if appropriate)
86256-APHTS (if appropriate)
84182-AN1BS (if appropriate)
86256 AN1TS (if appropriate)
84182-AN2BS (if appropriate)
86256 AN2TS (if appropriate)
86256 AN3TS (if appropriate)
86256 CRMTS (if appropriate)
86255-CS2CS (if appropriate)
84182-CRMWS (if appropriate)
86255-LG1CS (if appropriate)
84182-PC1BS (if appropriate)
86256 PC1TS (if appropriate)
86256 PC2TS (if appropriate)
84182-PCTBS (if appropriate)
86256 PCTTS (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
PAVAL	Paraneoplastic Autoantibody Eval, S	43104-9

Result ID	Test Result Name	Result LOINC® Value
89080	AGNA-1, S	84927-3
81722	Amphiphysin Ab, S	72327-0
80150	ANNA-1, S	33615-6
80776	ANNA-2, S	43187-4
83137	ANNA-3, S	43102-3
81185	P/Q-Type Calcium Channel Ab	94349-8
83077	CRMP-5-IgG, S	72504-4
29347	Interpretive Comments	57771-8
618905	IFA Notes	48767-8
83138	PCA-2, S	84925-7
9477	PCA-1, S	84924-0
83076	PCA-Tr, S	84926-5
89165	Neuronal (V-G) K+ Channel Ab, S	97560-7