MAYO CLINIC LABORATORIES

Test Definition: SPPC

Stiff-Person Spectrum Disorders Evaluation, including Progressive Encephalomyelitis with Rigidity and Myoclonus, Spinal Fluid

Overview

Useful For

Evaluating patients with suspected stiff-person syndrome (classical or focal forms, such as stiff-limb or stiff-trunk) and progressive encephalomyelitis with rigidity and myoclonus using spinal fluid specimens

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
SPPCI	Stiff-Person/PERM Interp, CSF	No	Yes
AMPHC	Amphiphysin Ab, CSF	No	Yes
DPPCC	DPPX Ab CBA, CSF	No	Yes
GD65C	GAD65 Ab Assay, CSF	Yes	Yes
GLYCC	Glycine Alpha1 LCBA, CSF	Yes	Yes

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
AMIBC	Amphiphysin Immunoblot, CSF	No	No
DPPTC	DPPX Ab IFA Titer, CSF	No	No
APHTC	Amphiphysin Ab Titer, CSF	No	No

Testing Algorithm

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

If the dipeptidyl-peptidase-like protein-6 (DPPX) cell binding assay result is positive, then DPPX antibody IFA titer will be performed at an additional charge.

Method Name

AMPHC, APHTC, DPPTC: Indirect Immunofluorescence Assay (IFA)
AMIBC: Immunoblot
DPPCC: Cell-Binding Assay (CBA)
GD65C: Radioimmunoassay (RIA)
GLYCC: Live Cell-Binding Assay (CBA)
SPPCI: Medical Interpretation

NY State Available

Yes

Specimen

Specimen Type
CSF

Ordering Guidance
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.

Multiple neurological phenotype-specific autoimmune/paraneoplastic evaluations are available. For more information as well as phenotype-specific testing options, refer to Autoimmune Neurology Test Ordering Guide.

For a list of antibodies performed with each evaluation, see Autoimmune Neurology Antibody Matrix.

Necessary Information
Provide the following information:
-Relevant clinical information
-Ordering healthcare professional's name, phone number, mailing address, and email address

Specimen Required
Container/Tube: Sterile vial
Preferred: Collection vial number 1
Acceptable: Any collection 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.

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

Stiff-person spectrum disorders include classical stiff-person syndrome, focal stiff-person forms (stiff-limb and stiff-trunk), and a severe encephalomyelitic form known as progressive encephalomyelitis with rigidity and myoclonus (PERM). Paraneoplastic and idiopathic autoimmune causes may be differentiated by a neuronal IgG antibody profile. The unifying clinical and electrophysiologic characteristic is central nervous system hyperexcitability. Clinical manifestations include stiffness, spasms, heightened startle responses, and falls. For the classical stiff-person form, the low back and lower extremities are principally affected. The stiff-limb phenotype may affect one or more limbs without truncal involvement. Truncal manifestations include low back spasms and deformity with sudden chest wall spasms and breathing difficulties. In addition, patients with PERM have encephalopathy (often with seizures), myoclonus (muscle jerking), and dysautonomia. The most common IgG biomarker detected in stiff-person spectrum is glutamic acid decarboxylase 65 (GAD65) antibody. These patients generally have a classical or limited stiff-person form, almost always have antibody values above 20.0 nmol/L, have accompanying nonneurological autoimmune disease in 50% (type 1 diabetes and thyroid disease being most common), and almost always without accompanying cancer. Amphiphysin-IgG positivity is most frequently encountered in patients with occult breast adenocarcinoma presenting with limb stiffness and spasms; neurogenic changes are usually detectable on clinical exam and electromyography. Patients with glycine receptor (GlyR [alpha1 1 subunit]) autoimmunity present more commonly with PERM or stiff-limb phenotype rather than the classical stiff-person form. Associated neoplasms in patients who are GlyR antibody positive include thymoma, but a general search for age- and sex-pertinent cancers should also be undertaken. Dipeptidyl-peptidase-like protein-6 (DPPX) antibody is associated with diverse central and autonomic presentations, including PERM. B-cell blood dyscrasias should be tested for in DPPX-IgG positive cases. All patients with stiff-person spectrum, both seropositive and seronegative, may be immune therapy responsive. GlyR-IgG may be predictive of immune therapy response, including in patients with coexisting GAD65 antibody.

Reference Values

Test ID	Reporting name	Methodology*	Reference value
SPPCI	Stiff-Person/PERM Interp, CSF	Medical interpretation	Interpretive report
AMPHC	Amphiphysin Ab, CSF	IFA	Negative
DPPCC	DPPX Ab CBA, CSF	CBA	Negative

GD65C	GAD65 Ab Assay, CSF	RIA	< or =0.02 nmol/L Reference values apply to all ages.
GLYCC	Glycine Alpha1 LCBA, CSF	LCBA	Negative

Reflex information

Test ID	Reporting name	Methodology	Reference value
AMIBC	Amphiphysin Immunoblot, CSF	IB	Negative
APHTC	Amphiphysin Ab Titer, CSF	IFA	<1:2
DPPTC	DPPX Ab IFA Titer, CSF	IFA	<1:2

*Methodology abbreviations:
Immunofluorescence assay (IFA)
Cell-binding assay (CBA)
Radioimmunoassay (RIA)
Live Cell-Binding Assay (LCBA)
Immunoblot (IB)

Neuron-restricted patterns of IgG staining that do not fulfill criteria for amphiphysin antibody may be reported as "unclassified antineuronal IgG." Complex patterns that include nonneuronal elements may be reported as "uninterpretable."

Interpretation

Spinal fluid antibody positivity supports the clinical diagnosis of stiff-person spectrum disorder (classical stiff-person, stiff-limb, stiff-trunk, or progressive encephalomyelitis with rigidity and myoclonus). A paraneoplastic basis should be considered.

Cautions

Negative results do not exclude the diagnosis of stiff-person spectrum disorder or progressive encephalomyelitis with rigidity and myoclonus (PERM). Glutamic acid decarboxylase 65 (GAD65) antibody positive values below 20 nmol/L should be interpreted with caution. Lower values are encountered in 8% of the general population. However, GAD65 autoimmunity (any antibody value) is associated with other autoimmune diseases that can cause neurological symptoms, including type 1 diabetes, pernicious anemia, hypothyroidism, and adrenal insufficiency.

Clinical Reference

1. Hinson SR, Lopez-Chiriboga AS, Bower JH, et al. Glycine receptor modulating antibody predicting treatable stiff-person spectrum disorders. Neurol Neuroimmunol Neuroinflamm. 2018;5(2):e438
2. Hutchinson M, Waters P, McHugh J, et al. Progressive encephalomyelitis, rigidity, and myoclonus: a novel glycine receptor antibody. Neurology. 2008;71(16):1291-1292
3. Martinez-Hernandez E, Arino H, McKeon A, et al. Clinical and immunologic investigations in patients with stiff-person spectrum disorder. JAMA Neurol. 2016;73(6):714-720
4. McKeon A, Martinez-Hernandez E, Lancaster E, et al. Glycine receptor autoimmune spectrum with stiff-man syndrome phenotype. JAMA Neurol. 2013;70(1):44-50
5. McKeon A, Robinson MT, McEvoy KM, et al. Stiff-man syndrome and variants: clinical course, treatments, and

outcomes. Arch Neurol. 2012;69(2):230-238
6. Pittock SJ, Lucchinetti CF, Parisi JE, et al. Amphiphysin autoimmunity: paraneoplastic accompaniments. Ann Neurol. 2005;58(1):96-107
7. Pittock SJ, Yoshikawa H, Ahlskog JE, et al. Glutamic acid decarboxylase autoimmunity with brainstem, extrapyramidal, and spinal cord dysfunction. Mayo Clin Proc. 2006;81(9):1207-1214
8. Tobin WO, Lennon VA, Komorowski L, et al. DPPX potassium channel antibody: frequency, clinical accompaniments, and outcomes in 20 patients. Neurology. 2014;83(20):1797-1803
9. 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;73(12):1161-1166

Performance

Method Description

Cell-Binding Assay:
Patient sample is applied to a composite slide containing transfected and nontransfected EU90 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)

Indirect Immunofluorescence Assay:
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.(Honorat 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)

Live Cell-Binding Assay:
This assay utilizes the T-REx System (Thermo Fisher). Expression of the glycine receptor alpha-1-subunit is repressed in the absence of tetracycline or doxycycline and induced in the presence of tetracycline or doxycycline. HEK293 cells stably expressing the tetracycline repressor and stably transfected with a plasmid encoding the alpha-1-subunit of the glycine receptor, under control of doxycycline, are grown in wells of a chamber slide. Twenty-four hours prior to the assay, the wells on the bottom half of the slide are treated with culture media, including doxycycline. After 24 hours, patient sample will be added to the living HEK293 cells held on ice. Bound IgG will be detected using a fluorophore conjugated anti-human IgG secondary antibody. Patient samples with IgG-specific antibodies for the glycine receptor will be positive on doxycycline-treated cells and negative on cells not treated with doxycycline.(Unpublished Mayo method)

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.(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 type 1 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;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 sample (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 samples will bind to the purified recombinant antigen, and negative samples will not bind. Strips are washed to remove unbound antibodies and then incubated with anti-human IgG antibodies (alkaline phosphatase-labeled) 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;6[3]:e552. doi:10.1212/NXI.0000000000000552)

PDF Report
No

Day(s) Performed
Profile tests: Monday through Sunday; Reflex tests: Varies

Report Available
5 to 10 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 2
86341
0431U
84182 (if appropriate)
86256 (if appropriate)
86256 (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
SPPC	Stiff-Person/PERM Eval, CSF	94712-7

Result ID	Test Result Name	Result LOINC® Value
5906	Amphiphysin Ab, CSF	90815-2
21702	GAD65 Ab Assay, CSF	94359-7
64934	DPPX Ab CBA, CSF	94283-9
606973	Glycine Alpha1 LCBA, CSF	96497-3
614602	Stiff-Person/PERM Interp, CSF	69048-7
618909	IFA Notes	48767-8