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

Testing Algorithm

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

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

Method Name

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

NY State Available

Yes

Specimen

Specimen Type
CSF

Ordering Guidance
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.

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: 3 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	
	Frozen	28 days	

	Ambient	72 hours	

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, and sudden chest wall spasms with breathing difficulties. Patients with PERM, in addition, 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 non-neurological autoimmune disease in 50% (type 1 diabetes and thyroid disease being most common), and almost always without accompanying cancer. Amphiphysin-IgG positivity is most commonly encountered in patients with occult breast adenocarcinoma presenting with limb stiffness and spasms; neurogenic changes are usually also detectable on clinical exam and electromyography. Glycine receptor (GlyR [alpha1 subunit]) autoimmunity patients present more commonly with PERM or stiff-limb phenotype, rather than the classical stiff-person form. Associated neoplasms in GlyR antibody positive patients 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 stiff-person spectrum patients, 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

AMPHIPHYSIN ANTIBODY
<1:2

DIPEPTIDYL-PEPTIDASE-LIKE PROTEIN-6 ANTIBODY CELL BINDING ASSAY (CBA)
Negative

GLUTAMIC ACID DECARBOXYLASE 65 ANTIBODY ASSAY
=0.02 nmol/L

GLYCINE RECEPTOR ALPHA1 IgG, CBA
Negative

AMPHIPHYSIN IMMUNOBLOT
Negative

DIPEPTIDYL-PEPTIDASE-LIKE PROTEIN IMMUNOFLUORESCENCE TITER
<1:2

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:e438
2. Hutchinson M, Waters P, McHugh J, et al: Progressive encephalomyelitis, rigidity, and myoclonus: a novel glycine receptor antibody. Neurology. 2008;71:1291-1292
3. Martinez-Hernandez E, Arino H, McKeon A, et al: Clinical and immunologic investigations in patients with stiff-person spectrum disorder. JAMA Neurol. 2016;73:714-720
4. McKeon A, Martinez-Hernandez E, Lancaster E, et al: Glycine receptor autoimmune spectrum with stiff-man syndrome phenotype. JAMA Neurol. 2013;70:44-50
5. McKeon A, Robinson MT, McEvoy KM, et al: Stiff-man syndrome and variants: clinical course, treatments, and outcomes. Arch Neurol. 2012 Feb;69(2):230-8
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;81:1207-1214
8. Tobin WO, Lennon VA, Komorowski L, et al: DPPX potassium channel antibody: frequency, clinical accompaniments, and outcomes in 20 patients. Neurology. 2014;83:1797-1803
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 December;73(12):1161-1166

Performance

Method Description

Indirect Immunofluorescence Assay:
The patient's sample is tested by a standardized immunofluorescence assay (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.(Pittock SJ, Kryzer TJ, Lennon VA: Paraneoplastic antibodies coexist and predict cancer, not neurological syndrome. Ann Neurol. 2004;56:715-719; 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 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.(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 Nov;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 specimen (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)

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, 02/2019)

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)

PDF Report
No

Day(s) Performed
AMPHC, DPPTC, GD65C:
Monday through Sunday

AMIBC:
Monday through Friday

DPPCC:
Monday through Thursday, Sunday

GLYCC:
Thursday

Report Available
5 to 10 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 Regional Manager. 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. This test has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

86255 x 3
86341
84182-AMIBC (if appropriate)
86256-DPPTC (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	94354-8
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