MAYO CLINIC LABORATORIES

Test Definition: GID2

Gastrointestinal Dysmotility, Autoimmune/Paraneoplastic Evaluation, Serum

Overview

Useful For

Investigating unexplained weight loss, early satiety, anorexia, nausea, vomiting, constipation, or diarrhea in a patient with a past or family history of cancer or autoimmunity

Directing a focused search for cancer

Investigating gastrointestinal symptoms that appear in the course or wake of cancer therapy, not explainable by recurrent cancer, metastasis, or therapy; detection of autoantibodies on this profile helps differentiate autoimmune gastrointestinal dysmotility from the effects of chemotherapy

Detecting early evidence of cancer recurrence in previously seropositive patients who have a rising titer of 1 or more autoantibodies

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
AGIDI	GI Dysmotility, Interpretation, S	No	Yes
AAGFS	AChR Alpha3 Ganglionic Flow, S	No	Yes
ANN1S	Anti-Neuronal Nuclear Ab, Type 1	No	Yes
APBIS	AP3B2 IFA, S	No	Yes
CS2CS	CASPR2-IgG CBA, S	No	Yes
CRMS	CRMP-5-IgG, S	No	Yes
DPPCS	DPPX Ab CBA, S	No	Yes
LG1CS	LGI1-IgG CBA, S	No	Yes
PCAB2	Purkinje Cell Cytoplasmic Ab Type 2	No	Yes

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
AN1BS	ANNA-1 Immunoblot, S	No	No
AN2BS	ANNA-2 Immunoblot, S	No	No
DPPTS	DPPX Ab IFA Titer, S	No	No
AN1TS	ANNA-1 Titer, S	No	No
APBCS	AP3B2 CBA, S	No	No

APBTS	AP3B2 IFA Titer, S	No	No
CRMTS	CRMP-5-IgG Titer, S	No	No
PC2TS	PCA-2 Titer, S	No	No
AAGTS	AChR Alpha3 Gang Flow Titer, S	No	No
MP1BS	MAP1B Immunoblot, S	No	No
CRMWS	CRMP-5-IgG Western Blot, S	No	No

Testing Algorithm

If the ganglionic acetylcholine receptor (alpha 3) flow screen assay result requires further investigation, then the alpha3 flow titration assay will be performed at an additional charge.

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

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

If the IFA pattern suggests AP3B2 (adaptor protein 3 beta2) antibodies, then the AP3B2 cell binding assay (CBA) and AP3B2 titer will be performed at an additional charge.

If the IFA pattern suggests Purkinje cell cytoplasmic antibody type 2 (PCA-2), then the PCA-2 IFA titer and microtubule-associated protein 1B (MAP1B) immunoblot will be performed at an additional charge.

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

For more information see Autoimmune/Paraneoplastic Gastrointestinal Dysmotility Evaluation Algorithm.

Special Instructions

- Autoimmune/Paraneoplastic Gastrointestinal Dysmotility Evaluation Algorithm

Method Name

AGIDI: Technical Interpretation
ANN1S, APBIS, CRMS, PCAB2, AN1TS, APBTS, CRMTS, DPPTS, PC2TS: Indirect Immunofluorescence Assay (IFA)
CS2CS, DPPCS, LG1CS, APBCS: Cell Binding Assay (CBA)
CRMWS: Western Blot (WB)
AN1BS, AN2BS, MP1BS: Immunoblot (IB)
AAGFS, AAGTS: Flow Cytometry

NY State Available

Yes

Specimen

Specimen Type
Serum

Ordering Guidance
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.

When more than one evaluation is ordered on the same order number the duplicate will be canceled.

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

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 for 1 week and assayed if sufficiently decayed or canceled if radioactivity remains.

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

Specimen Required
Patient Preparation: For optimal antibody detection, collect specimen before starting immunosuppressant medication or intravenous immunoglobulin (IVIg) 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 Serum
Collection Instructions: Centrifuge and aliquot serum into a plastic vial.

Forms
If not ordering electronically, complete, print, and send Gastroenterology and Hepatology Test Request (T728) with the specimen

Specimen Minimum Volume

Serum: 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	
	Ambient	72 hours	
	Frozen	28 days	

Clinical & Interpretive

Clinical Information

Autoimmune gastrointestinal dysmotility (AGID) is a limited form of dysautonomia (also known as autoimmune autonomic ganglionopathy or neuropathy) that is sometimes a paraneoplastic disorder. Neoplasms most frequently found are lung cancer, thymoma, and miscellaneous adenocarcinomas. Diagnosis is confirmed by objective abnormalities on gastrointestinal (GI) motility studies (eg, gastric, small intestinal, or colonic nuclear transit studies; esophageal, gastroduodenal, or colonic manometry or anorectal manometry with balloon expulsion). These disorders target autonomic postganglionic synaptic membranes and, in some cases, ganglionic neurons and autonomic nerve fibers, and may be accompanied by sensory small fiber neuropathy. Onset may be subacute or insidious. There may be additional manifestations of dysautonomia (eg, impaired pupillary light reflex, anhidrosis, orthostatic hypotension, sicca manifestations, and bladder dysfunction) or signs of other neurologic impairment. Autonomic reflex testing and a thermoregulatory sweat test are valuable aids in the documentation of objective abnormalities.

The serological profile of AGID may include autoantibodies specific to onconeural proteins found in the nucleus, cytoplasm, or plasma membrane of neurons or muscle. Some of these autoantibodies are highly predictive of an underlying cancer. A commonly encountered autoantibody marker of AGID is the ganglionic neuronal alpha-3-acetylcholine receptor (alpha-3-AChR) autoantibody. The pathogenicity of this autoantibody was demonstrated in rabbits immunized with a recombinant extracellular fragment of the alpha-3-AChR subunit and in mice injected with IgG from high-titered alpha-3-AChR autoantibody-positive rabbit or human sera.

Importantly, cancer is detected in 30% of patients with alpha-3-AChR autoantibody. Cancer risk factors include the patient's previous or family history of cancer, history of smoking, or social and environmental exposure to carcinogens. Early diagnosis and treatment of the neoplasm favor less morbidity from the GI dysmotility disorder. The cancers

recognized most frequently with alpha-3-AChR autoantibody include lymphoma and adenocarcinomas of breast, lung, prostate, and GI tract. A specific neoplasm is often predictable when a patient's autoantibody profile includes other autoantibodies to onconeural proteins shared by neurons, glia, or muscle. Small-cell lung carcinoma is found in 80% of patients who are antineuronal nuclear antibody-type 1 (ANNA-1, also known as anti-Hu) positive, and 23% of patients who are ANNA-1 positive have GI dysmotility. The most common GI manifestation is gastroparesis, but the most dramatic is pseudoobstruction.

Reference Values

Reference Values

Test ID	Reporting Name	Methodology*	Reference Value
AGIDI	GI Dysmotility, Interpretation, S	Technical interpretation	Interpretive report
AAGFS	AChR Alpha3 Ganglionic Flow, S	FC	Negative
ANN1S	Anti-Neuronal Nuclear Ab, Type 1	IFA	Negative
APBIS	AP3B2 IFA, S	IFA	Negative
CS2CS	CASPR2-IgG CBA, S	CBA	Negative
CRMS	CRMP-5-IgG, S	IFA	Negative
DPPCS	DPPX Ab CBA, S	CBA	Negative
LG1CS	LGI1-IgG CBA, S	CBA	Negative
PCAB2	Purkinje Cell Cytoplasmic Ab Type 2	IFA	Negative

Reflex Information:

Test ID	Reporting Name	Methodology*	Reference Value
AAGTS	AChR Alpha3 Gang Flow Titer, S	FC	<1:10
AN1BS	ANNA-1 Immunoblot, S	IB	Negative
AN1TS	ANNA-1 Titer, S	IFA	<1:240
AN2BS	ANNA-2 Immunoblot, S	IB	Negative
APBCS	AP3B2 CBA, S	CBA	Negative
APBTS	AP3B2 IFA Titer, S	IFA	<1:240
CRMTS	CRMP-5-IgG Titer, S	IFA	<1:240
CRMWS	CRMP-5-IgG Western Blot, S	WB	Negative
DPPTS	DPPX Ab IFA Titer, S	IFA	<1:240
MP1BS	MAP1B Immunoblot, S	IB	Negative
PC2TS	PCA-2 Titer, S	IFA	<1:240

*Methodology abbreviations used:
Cell-binding assay (CBA)
Flow cytometry (FC)
Immunoblot (IB)
Immunofluorescence assay (IFA)
Western blot (WB)

Interpretation

Antibodies directed at onconeural proteins shared by neurons, 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 signs and symptoms. It is not uncommon for more than one antibody to be detected. Three classes of antibodies are recognized (the individual antibodies from each class included in the profile are denoted in parentheses):
-Antineuronal nuclear autoantibody-type 1 (ANNA-1)
-Neuronal and muscle cytoplasmic (collapsin response-mediator protein-5: CRMP5)
-Plasma membrane cation channel (neuronal ganglionic alpha-3-acetylcholine receptor: AChR); These autoantibodies are potential effectors of autoimmune gastrointestinal dysmotility.

Cautions

Negative results do not exclude autoimmune gastrointestinal dysmotility or cancer.

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

Clinical Reference

1. Flanagan EP, Saito YA, Lennon VA, et al. Immunotherapy trial as diagnostic test in evaluating patients with presumed autoimmune gastrointestinal dysmotility. Neurogastroenterol Motil. 2014;26(9):1285-1297. doi:10.1111/nmo.12391
2. Dhamija R, Tan KM, Pittock SJ, Foxx-Orenstein A, Benarroch E, Lennon VA. Serologic profiles aiding the diagnosis of autoimmune gastrointestinal dysmotility. Clin Gastroenterol Hepatol. 2008;6(9):988-992. doi:10.1016/j.cgh.2008.04.009
3. Cutsforth-Gregory JK, McKeon A, Coon EA, et al. Ganglionic antibody level as a predictor of severity of autonomic failure. Mayo Clin Proc. 2018;93(10):1440-1447. doi:10.1016/j.mayocp.2018.05.033
4. 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. doi:10.1212/WNL.0000000000000991

Performance

Method Description

Indirect Immunofluorescence Assay:
The patient's sample is tested by a standardized immunofluorescence assay 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;4[5]:e385. doi:10.1212/NXI.0000000000000385)

Flow Cytometry:
This assay is a live cell-based binding assay using flow cytometry to detect ganglionic acetylcholine receptor (AChR) binding antibodies in patient serum. Human embryonic kidney cells (HEK293) transiently expressing human alpha-3 beta-4 ganglionic AChR subunits, along with accessory proteins required for receptor expression, are used as the antigen source. Receptor expression is enhanced prior to analysis under controlled culture conditions. Cells are incubated with diluted patient serum to allow binding of AChR-specific antibodies to surface-expressed receptors. Bound human IgG is

detected using a fluorophore-conjugated anti-human IgG secondary antibody, with a viability dye included to exclude non-viable cells. Fluorescence intensity is measured by flow cytometry, and antibody binding is assessed by comparing signal from AChR-expressing cells to control cells lacking receptor expression. A humanized AChR-specific monoclonal antibody is used as an assay calibrator. The IgG Binding Index (IBI) of patient samples is compared to the assay calibrator to calculate a Cut-off Index (COI). Patient samples with a COI = 1.0 are reported as positive. Positive samples are further titrated to determine the end-point dilution, defined as the highest serum dilution that remains above the calibrator.(Unpublished Mayo method)

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;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)

Immunoblot for ANNA-1 and ANNA-2:
All steps are performed at ambient temperature (18-28 degrees C) utilizing the EUROBlot One instrument. Diluted patient sample 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 antihuman IgG antibodies (alkaline phosphatase-labeled) for 30 minutes. The strips are again washed to remove unbound antihuman IgG antibodies and nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate (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)

Immunoblot for MAP1B:
Test strips are incubated with appropriately diluted sample, prepared to meet the assay's required end-dilution for each specimen type. Samples and controls are added directly to the strips, ensuring they are fully covered with dilution buffer, then mixed gently and incubated for 60 minutes at room temperature on a rocking platform. After incubation, the strips undergo a series of wash steps using diluted wash buffer, followed by a 30-minute incubation with a ready-to-use alkaline phosphatase-labeled IgG conjugate. The strips are washed again using the same procedure, then incubated with a ready-to-use substrate solution for 20 minutes at room temperature. The reaction is stopped by rinsing with distilled water through several wash cycles. Strips are then placed on absorbent material and allowed to dry fully, generally requiring at least two hours. Results are interpreted only after the strips have dried completely, and dried strips are stored protected from light.(Package insert: PCA-2 line assay. ravo Diagnostika GmbH; 05/2023

Cell-Binding Assay:
Patient sample is applied to a composite slide containing transfected and nontransfected cells. After incubation and

washing, fluorescein-conjugated goat-antihuman IgG is applied to detect the presence of patient IgG binding.(Klein CJ, Lennon VA, Aston PA, et al. Insights From LGI1 and CASPR2 potassium channel complex autoantibody subtyping. JAMA Neurol 2013;70[2]:229-234; package insert: IIFT: Neurology Mosaics, Instructions for the indirect immunofluorescence test. EUROIMMUN; FA_112d-1_A_UK_C13, 02/2019)

PDF Report
No

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

Report Available
8 to 12 days

Specimen Retention Time
2 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 8
86256 AAGTS (if appropriate)
84182 AN1BS (if appropriate)
86256 AN1TS (if appropriate)
84182 AN2BS (if appropriate)
86255 APBCS (if appropriate)
86256 APBTS (if appropriate)
86256 CRMTS (if appropriate)
84182 CRMWS (if appropriate)
86256 DPPTS (if appropriate)

84182 MP1BS (if appropriate)
86256 PC2TS (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
GID2	GI Dysmotility, Autoimm/Paraneo, S	97557-3

Result ID	Test Result Name	Result LOINC® Value
80150	ANNA-1, S	33615-6
83077	CRMP-5-IgG, S	72504-4
83138	PCA-2, S	84925-7
34269	GI Dysmotility, Interpretation, S	69048-7
618899	IFA Notes	48767-8
64279	LGI1-IgG CBA, S	94287-0
64281	CASPR2-IgG CBA, S	94285-4
64933	DPPX Ab CBA, S	94676-4
615863	AP3B2 IFA, S	101907-4
622854	AChR Alpha3 Ganglionic Flow, S	In Process