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 |
| GANG | AChR Ganglionic Neuronal Ab, 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 |
| CRMWS | CRMP-5-IgG Western Blot, S | Yes | 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 |

## Testing Algorithm

If client requests or 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 (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 AP3B22 cell binding assay (CBA) and AP3B22 titer will be performed at an additional charge.

If the IFA pattern suggests PCA-2 antibody, then the PCA-2 IFA titer 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: Medical Interpretation

ANN1S, AN1TS, APBIS, APBTS, DPPTS, CRMS, CRMTS, PCAB2, PC2TS: Indirect Immunofluorescence Assay (IFA)

APBCS, CS2CS, LG1CS, DPPCS: Cell-Binding Assay (CBA)

CRMWS: Western Blot (WB)

AN1BS, AN2BS: Immunoblot (IB)

GANG: Radioimmunoassay (RIA)

## 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 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, specimen collection is recommended 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
**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
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 for 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. A direct relationship between antibody titer and severity of dysautonomia occurs in both experimental animals and patients. Patients with high alpha-3-AChR autoantibody values (>1.0 nmol/L) generally present with profound pandysautonomia, and those with lower alpha-3-AChR autoantibody values may have limited autoimmune dysautonomia or other neurological signs and symptoms.

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

| Test ID | Reporting Name | Methodology* | Reference Value |
|---|---|---|---|
| AGIDI | GI Dysmotility, Interpretation, S | Medical interpretation | Interpretive report |
| GANG | AChR Ganglionic Neuronal Ab, S | RIA | < or =0.02 nmol/L |
| 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 |
|---|---|---|---|
| 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 |
| PC2TS | PCA-2 Titer, S | IFA | <1:240 |

*Methodology abbreviations used:
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 ANNA-1, CRMP-5-IgG, or PCA-2 may be reported as "unclassified anti-neuronal IgG." Complex patterns that include nonneuronal elements may be reported as "uninterpretable."

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 for 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, 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 [ACh] receptor).
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 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)

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 liter 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; 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)

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, Kim K, Benarroch EE, Lennon VA l. 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:
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 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 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)

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)

### PDF Report
No

### Day(s) Performed

Profile tests: Monday through Sunday; Reflex tests: Varies

### Report Available
8 to 12 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
83519
86255 x 7
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)
86255 DPPCS (if appropriate)
86256 DPPTS (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 |
| 84321 | AChR Ganglionic Neuronal Ab, S | 94694-7 |
| 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 |