MAYO CLINIC LABORATORIES

Test Definition: SCNGP

Congenital Neutropenia, Primary Immunodeficiency Disorder Panel (18 genes), Next-Generation Sequencing, Varies

Overview

Useful For

Providing a comprehensive genetic evaluation for patients with a personal or family history suggestive of congenital neutropenia, cyclic neutropenia, or other primary immunodeficiency disorder (PIDD) presenting with significant neutropenia

Establishing a diagnosis and, in some cases, allowing for appropriate management and surveillance for disease features based on the gene involved

Identifying variants within genes known to be associated with PIDD characterized by significant neutropenia allowing for predictive testing of at-risk family members

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CULFB	Fibroblast Culture for Genetic Test	Yes	No

Genetics Test Information

This test includes next-generation sequencing and supplemental Sanger sequencing to test for variants in the *AP3B1*(*HP2*), *CSF3R, CXCR4, ELANE*(*ELA2*), *G6PC3, GATA2, GFI1, HAX1, LAMTOR2*(*MAPBPIP*), *RAC2, SBDS, SLC37A4, TAZ, USB1(C16ORF57*), *VPS13B*(*COH1*), *VPS45, WAS,* and *WIPF1* genes.

Identification of a pathogenic variant may assist with prognosis, clinical management, familial screening, and genetic counseling.

Testing Algorithm

For skin biopsy or cultured fibroblast specimens, fibroblast culture testing will be performed at an additional charge. If viable cells are not obtained, the client will be notified.

Special Instructions

- Informed Consent for Genetic Testing
- Blood Spot Collection Card-Spanish Instructions
- Primary Immunodeficiencies Patient Information
- Blood Spot Collection Card-Chinese Instructions
- Informed Consent for Genetic Testing (Spanish)
- Blood Spot Collection Instructions

Method Name

Custom Sequence Capture and Targeted Next-Generation Sequencing followed by Polymerase Chain Reaction (PCR) and

Supplemental Sanger Sequencing

NY State Available
Yes

Specimen

Specimen Type
Varies

Ordering Guidance
Targeted testing for familial variants (also called site-specific or known mutations testing) is available for the genes on this panel. See FMTT / Familial Mutation, Targeted Testing, Varies.

Necessary Information
1. **Primary Immunodeficiencies Patient Information** (T791) is strongly recommended, but not required, to be filled out and sent with the specimen. This information aids in providing a more thorough interpretation of test results. Ordering providers are strongly encouraged to complete the form and send it with the specimen.
2. Include physician name and phone number with specimen.

Specimen Required
Patient Preparation: A previous bone marrow transplant from an allogenic donor or a recent (ie, <6 weeks from time of sample collection) heterologous blood transfusion will interfere with testing. Call 800-533-1710 for instructions for testing patients who have received a bone marrow transplant.

Submit only 1 of the following specimens:

Preferred:
Specimen Type: Whole blood
Container/Tube: Lavender top (EDTA)
Specimen Volume: 5 mL
Collection Instructions:
1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot.**
Specimen Stability Information: Ambient (preferred)4 days/Refrigerated 14 days
Additional Information: For patients with severe neutropenia, DNA yield may be insufficient for testing. Consider sending additional volume or an alternate specimen type.

Specimen Type: Blood spot
Supplies: Card-Blood Spot Collection Filter Paper (T493)
Container/Tube:

Preferred: Collection card (Whatman Protein Saver 903 Paper)
Acceptable: Whatman FTA Classic paper, PerkinElmer 226 (formerly Ahlstrom 226) filter paper, or blood spot collection card
Specimen Volume: 5 Blood spots
Collection Instructions:
1. An alternative blood collection option for a patient 1 year of age or older is a fingerstick. For infants younger than 1 year, a heel stick should be used. See Dried Blood Spot Collection Tutorial for how to collect blood spots via fingerstick.
2. Let blood dry on the filter paper at ambient temperature in a horizontal position for a minimum of 3 hours.
3. Do not expose specimen to heat or direct sunlight.
4. Do not stack wet specimens.
5. Keep specimen dry.
Specimen Stability Information: Ambient (preferred)/Refrigerated
Additional Information:
1. For patients with severe neutropenia, DNA yield may be insufficient for testing. Consider sending additional volume or an alternate specimen type.
2. For collection instructions, see Blood Spot Collection Instructions.
3. For collection instructions in Spanish, see Blood Spot Collection Card-Spanish Instructions (T777).
4. For collection instructions in Chinese, see Blood Spot Collection Card-Chinese Instructions (T800).

Specimen Type: Peripheral blood mononuclear cells (PBMC)
Container/Tube: Cell pellet
Collection Instructions: Send as a suspension in freezing medium or cell pellet frozen on dry ice.
Specimen Stability Information: Frozen

Specimen Type: Cultured fibroblasts
Container/Tube: T-75 or T-25 flask
Specimen Volume: 1 Full T-75 or 2 full T-25 flasks
Specimen Stability Information: Ambient (preferred)/Refrigerated <24 hours
Additional Information: Indicate the tests to be performed on the fibroblast culture cells. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks is required to culture fibroblasts before genetic testing can occur.

Specimen Type: Skin biopsy
Supplies: Fibroblast Biopsy Transport Media (T115)
Container/Tube: Sterile container with any standard cell culture media (eg, minimal essential media, RPMI 1640). The solution should be supplemented with 1% penicillin and streptomycin. Tubes of culture media can be supplied upon request (Eagle's minimum essential medium with 1% penicillin and streptomycin).
Specimen Volume: 4-mm punch
Specimen Stability Information: Refrigerated (preferred)/Ambient
Additional Information: A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing . An additional 3 to 4 weeks is required to culture fibroblasts before genetic testing can occur.

Specimen Type: Extracted DNA

Container/Tube: 2 mL screw top tube
Specimen Volume: 100 mcL (microliters)
Collection Instructions:
1. The preferred volume is 100 mcL at a concentration of 250 ng/mcL
2. Include concentration and volume on tube.
Specimen Stability Information: Frozen (preferred)/Ambient/Refrigerated

Forms

1. **New York Clients-Informed consent is required.** Document on the request form or electronic order that a copy is on file. The following documents are available in Special Instructions:
-Informed Consent for Genetic Testing (T576)
-Informed Consent for Genetic Testing-Spanish (T826)
2. Primary Immunodeficiencies Patient Information (T791) is recommended. See Special Instructions.

Specimen Minimum Volume

Whole blood: 1 mL

Reject Due To

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical & Interpretive

Clinical Information

Severe congenital neutropenia is a primary immunodeficiency disorder that is characterized by severe and recurrent bacterial infections, such as otitis media, bronchitis, pneumonia, osteomyelitis, and cellulitis, typically with the absence of pus at the infected site. Susceptibility to fungal infections may also be observed. Neutropenia may be an isolated finding or may be part of a syndrome. This panel includes genes associated with neutropenia as a major presenting feature; other panels may be more appropriate when neutropenia is identified but not as the main finding.

Pathogenic variants in *ELANE,* which encodes neutrophil elastase, can result in severe congenital neutropenia type 1 (SCN1) or cyclic neutropenia. SCN1 often presents immediately with omphalitis, while diarrhea, pneumonia, and deep abscesses affecting the liver, lungs, or subcutaneous tissues are noted within the first year. Patients are at risk for development of myelodysplastic syndrome or acute myelogenous leukemia, presumably due to acquired mutations in *CSF3R* (which may also be identified in the presence of congenital neutropenia due to variants in genes other than *ELANE*, see below). Biallelic mutations in *CSF3R* have also been recently reported to be associated with severe congenital neutropenia. Cyclic neutropenia typically presents in the first year of life with 3-week-long oscillations in cell counts along with intervals of fever, oral ulcerations, and ulcerations; between intervals, patients are generally healthy. Unlike

SCN1, cyclic neutropenia is not associated with risk of malignancy. Both SCN1 and cyclic neutropenia are inherited in an autosomal dominant pattern from an affected parent, although de novo variants have been identified. Studies have demonstrated pathogenic variants in *ELANE* in nearly 100% of cases with well-documented classical cyclic neutropenia, while in some cases with atypical presentations (ie, oscillations that are not 3 weeks) a variant in *ELANE* is not identified. *ELANE* variants are identified in 38% to 80% of cases of congenital neutropenia, depending on the criteria used to identify patients. Although there is some overlap, generally, variants at the active site of neutrophil elastase result in cyclic neutropenia, while variants that prevent normal folding or packaging of the enzyme cause congenital neutropenia.

In addition to variants in *ELANE*, severe congenital neutropenia, where the predominant finding is neutropenia, can be inherited as a result of pathogenic variants in other genes. Dominant variants in *GFI1* (encoding growth factor independent 1) result in severe congenital neutropenia type 2 (SCN2). Pathogenic variants in *G6PC3* (encoding glucose-6-phosphate 3), which are inherited in an autosomal recessive manner, can result in a phenotypic spectrum from isolated/nonsyndromic severe congenital neutropenia to classic G6PC3 deficiency (severe neutropenia along with cardiovascular and urogenital abnormalities) to severe G6PC3 deficiency (also known as Dursun syndrome, which includes features of classic G6PC3 deficiency along with severe lymphopenia, primary pulmonary hypertension, thymic hypoplasia, among other features). Kostmann disease or severe congenital neutropenia type 3 (SCN3) is due to recessive inheritance of pathogenic variants in *HAX1* (which encodes HCLS1-associated protein X-1) and may result in seizures and developmental delay in addition to neutropenia. Along with neutropenia, variants in *VPS45* inherited in an autosomal recessive manner (also known as severe congenital neutropenia type 5 [SNC5]) are associated with neutrophil dysfunction, bone marrow fibrosis, and nephromegaly due to renal extramedullary hematopoiesis. While loss-of-function variants in *WAS,* which is located on the X chromosome, cause Wiskott-Aldrich syndrome (characterized by thrombocytopenia, eczema, and recurrent infections), gain-of-function variants affecting the autoinhibitory structure of the protein, have been associated with congenital neutropenia, along with variable lymphopenia, decreased lymphocyte proliferation, and impaired phagocyte activity. Pathogenic variants in *WIPF1* can present with similar findings to Wiskott-Aldrich syndrome.

Severe neutropenia may also be present as part of a multisystem disorder. Barth syndrome, due to pathogenic variants in *TAZ*, which is located on the X-chromosome, is characterized by neutropenia, cardio- and skeletal myopathy, growth delay, and distinctive facial features. Biallelic variants in *C16orf57* manifest as poikiloderma with neutropenia; the neutropenia may be cyclical. In Cohen syndrome, an autosomal recessive disorder due to variants in *COH1* (also known as *VPS13B*), neutropenia is accompanied by hypotonia, developmental delays, microcephaly, failure to thrive in infancy, truncal obesity in adolescent years, ophthalmologic findings, joint hypermobility, a cheerful disposition, and characteristic facial features. Glycogen storage disease type I (GSDI), caused by biallelic pathogenic variants in either *G6PC* or *SLC37A4*, when untreated can result in chronic neutropenia and impaired neutrophil and monocyte function, as well as the characteristic findings that include accumulation of glycogen and fat in the liver and kidneys. Pathogenic variants in *LAMTOR2/MAPBPIP* have been shown to result in neutropenia, decreased cytotoxic activity of CD8+ T cells, short stature, and hypopigmented skin. Persistent or intermittent neutropenia is often a presenting feature of Shwachman-Diamond syndrome (SDS), which is also characterized by exocrine pancreatic dysfunction (with malabsorption, malnutrition, and growth failure), bone abnormalities, and hematologic abnormalities (single- or multilineage cytopenias along with predisposition to myelodysplastic syndrome and acute myelogenous leukemia). SDS is an autosomal recessive disorder due to pathogenic variants in *SBDS*. Warts, hypogammaglobulinemia, immunodeficiency, and myelokathexis (WHIM) syndrome is characterized by neutropenia in addition to hypogammaglobulinemia, and susceptibility to human papillomavirus. It is due to autosomal dominant pathogenic

variants in *CXCR4*. Although most forms of Hermansky-Pudlak syndrome do not include significant neutropenia, type 2 caused by variants in *AP3B1* can be associated with persistent neutropenia and increased infections in addition to the typical findings of tyrosinase-positive oculocutaneous albinism, platelet storage pool deficiency, pulmonary fibrosis, and granulomatous colitis. Few patients with *RAC2* pathogenic variants have been identified, but neutrophil dysfunction appears to be a feature, though CD11b expression and specific granule release appear to be preserved. Both individuals with dominant and individuals with recessive inheritance have been identified, with and without additional associated phenotypic findings.

GATA-binding protein (GATA2) deficiency demonstrates a wide spectrum of clinical presentations, including neutropenia. Most variants appear to arise de novo (spontaneously) and are then transmitted in an autosomal dominant manner. If the clinical phenotype strongly suggests GATA2 deficiency, this gene is available as a stand-alone test (see GATA2 / GATA-Binding Protein 2 [*GATA2]*, Full Gene, Next-Generation Sequencing, Varies). This panel **does not** evaluate for somatic (acquired) *ASXL1* variants associated with GATA2 deficiency.

Genes included in this test

Gene (alias)	Protein	OMIM	Incidence	Inheritance	Phenotype disorder
AP3B1	AP-3 complex subunit beta-1 isoform 1	603401	Rare	AR	Hermansky-Pudlak syndrome 2
CSF3R	Granulocyte colony-stimulating factor receptor isoform a precursor	138971		AR, acquired	Severe congenital neutropenia
CXCR4	C-X-C chemokine receptor type 4 isoform b	162643		AD	Myelokathexis, isolated, WHIM syndrome (AD)
ELANE	Neutrophil elastase preproprotein	130130	2:1,000,000-3:1,000,000 (SCN); 1:1,000,000 (cyclic neutropenia)	AD	Severe congenital neutropenia (SCN), cyclic neutropenia
G6PC3	Glucose-6-phosphatase 3	611045		AR	Dursun syndrome, severe congenital neutropenia (SCN) 4
GATA2	Endothelial transcription factor GATA-2 isoform 1	137295		AD	Immunodeficiency 21, Emberger syndrome,

					susceptibility to acute myeloid leukemia and myelodysplastic syndrome
GFI1	Zinc finger protein Gfi-1	600871		AD	Severe congenital neutropenia (SCN) 2(AD), nonimmune chronic idiopathic neutropenia of adults
HAX1	HCLS1-associated protein X-1 isoform a	605998		AR	Severe congenital neutropenia (SCN) 3
LAMTOR2 (MAPBPIP)	Ragulator complex protein LAMTOR2 isoform 1	610389		AR	Immunodeficiency due to defect in MAPBP-interacting protein
RAC2	ras-Related C3 botulinum toxin substrate 2	602049		AD/AR	Neutrophil functional defects
SBDS	Ribosome maturation protein SBDS	607444		AR	Shwachman-Diamond syndrome, susceptibility to aplastic anemia
SLC37A4	Ddipeptidyl peptidase 1 isoform a preproprotein	602671		AR	Glycogen storage disease Ib and 1c
TAZ	Tafazzin isoform 1	300394		XL	Barth syndrome
USB1 (C16ORF57)	U6 snRNA phosphodiesterase isoform 1	613276	Rare	AR	Poikiloderma with neutropenia
VPS13B (COH1)	Vacuolar protein sorting-associated protein 13B isoform 5	607817		AR	Cohen syndrome
VPS45	Vacuolar protein sorting-associated protein 45 isoform	610035		AR	Severe congenital neutropenia (SCN) 5

	1				
WAS (Gain-of-function mutations)	Wiskott-Aldrich syndrome protein	300392		XL (gain of function)	Neutropenia, severe congenital, X-linked, thrombocytopenia, X-linked
WIPF1	WAS/WASL-interacting protein family member 1	602357		In progress	Wiskott-Aldrich syndrome 2

AD=autosomal dominant
AR=autosomal recessive
XL=X-linked

Reference Values

An interpretive report will be provided.

Interpretation

Evaluation and categorization of variants is performed using the most recent published American College of Medical Genetics and Genomics (ACMG) recommendations as a guideline. Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

Multiple in silico evaluation tools may be used to assist in the interpretation of these results. The accuracy of predictions made by in silico evaluation tools is highly dependent upon the data available for a given gene, and predictions made by these tools may change over time. Results from in silico evaluation tools should be interpreted with caution and professional clinical judgment.

Cautions

The majority of DNA extracted from whole blood is derived from neutrophils. Therefore, blood specimens collected from patients with severe neutropenia may yield limited quantity of DNA that is insufficient for testing. When ordering this test for patients with severe neutropenia, please consider timing the collection when the patient has a higher neutrophil count, submitting an alternate specimen type, or collecting additional blood volume.

Clinical Correlations:
Some individuals who have involvement of one or more of the genes on the panel may have a variant that is not identified by the methods performed (eg, promoter variants, deep intronic variants). The absence of a variant, therefore, does not eliminate the possibility of disease. Test results should be interpreted in context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

For predictive testing of asymptomatic individuals, it is often useful to first test an affected family member. Identification of a pathogenic variant in an affected individual allows for more informative testing of at-risk individuals.

Technical Limitations:

Next-generation sequencing may not detect all types of genetic variants. The variant detection software has lower detection efficiency for insertion/deletion variants as compared to single nucleotide variants. Therefore, small deletions and insertions greater than 8 nucleotides in length may not be detected by this test. Copy Number variations (CNV) are not currently reported for any of the genes on this panel. Additionally, rare alterations (polymorphisms) may be present that could lead to false-negative or false-positive results. In some cases, DNA variants of undetermined significance may be identified. If results do not match clinical findings, consider alternative methods for analyzing these genes, such as Sanger sequencing or large deletion/duplication analysis. If the patient has had an allogeneic blood or bone marrow transplant or a recent (ie, <6 weeks from time of sample collection) heterologous blood transfusion, results may be inaccurate due to the presence of donor DNA. Call 800-533-1710 for instructions for testing patients who have received a bone marrow transplant.

Reclassification of Variants-Policy:
At this time, it is not standard practice for the laboratory to systematically review likely deleterious alterations or variants of uncertain significance that are detected and reported. The laboratory encourages health care providers to contact the laboratory at any time to learn how the status of a particular variant may have changed over time. Consultation with a healthcare provider, or team of healthcare providers, with expertise in genetics and primary immunodeficiencies, is recommended for interpretation of this result.

A list of benign and likely benign variants detected is available from the lab upon request.

Contact the laboratory if additional information is required regarding the transcript or human genome assembly used for the analysis of results.

Clinical Reference

1. Picard C, Gaspar HB, Al-Herz W, et al: International Union of Immunological Societies: 2017 Primary Immunodeficiency Disease Committee report on inborn errors of immunity, J Clin Immunol. 2018;38:96-128. doi: 10.1007/s10875-017-0464-9
2. Donadieu J, Fenneteau O, Beaupain B, Mahlaoui N, Bellanne Chantelot C: Congenital neutropenia: diagnosis, molecular bases and patient management. Orphanet J Rare Dis. 2011;6:26. doi: 10.1186/1750-1172-6-26
3. Boxer LA, Stein S, Buckley D, et al: Strong evidence for autosomal dominant inheritance of severe congenital neutropenia associated with ELA2 mutations. J Pediatr. 2006;148:633-636
4. Beel K, Bandenberghe P: G-CSF receptor (*CSF3R*) mutations in X-linked neutropenia evolving to acute myeloid leukemia or myelodysplasia. Haematologica. 2009;94(10):1449-1452
5. Klein C: Genetic defects in severe congenital neutropenia: emerging insights into life and death of human neutrophil granulocytes. Ann Rev Immunol. 2011;29:399-413
6. Albert MH, Notarangelo LD, Ochs HD: Clinical spectrum, pathophysiology and treatment of the Wiskott-Aldrich syndrome. Cur Opin Hematol. 2011;18(1):42-48
7. Concolino D, Roversi G, Muzzi GL, et al: Clericuzio-type poikiloderma with neutropenia syndrome in three sibs with mutations in the C16orf57 gene: delineation of the phenotype. Am J Med Genet. 2010;152A(10):2588-2594
8. Bohn G, Allroth A, Brandea G, et al: A novel human primary immunodeficiency syndrome caused by deficiency of endosomal adaptor protein p14. Nature Med. 2007;13(1):38-45
9. Triot A, Jarvinen PM, Arostegui JI, et al: Inherited biallelic CSF3R mutations in severe congenital neutropenia. Blood. 2014;123:3811-3817

10. Bousifiha AA, et al: A phenotypic approach for IUIS PID classification and diagnosis: guidelines for clinicians at the bedside. J Clin Immunol. 2013;Aug;33(6):1078-1087

Performance

Method Description

Next-generation sequencing (NGS) is performed using an Illumina instrument with paired-end reads. The DNA is prepared for NGS using a custom Agilent SureSelect Target Enrichment System. Data is analyzed with a bioinformatics software pipeline. Supplemental Sanger sequencing may be performed occasionally in regions where NGS is insufficient for data capture or not specific enough to correctly identify a variant.(Unpublished Mayo method)

Genes analyzed: AP3B1(HP2), CSF3R, CXCR4, ELANE(ELA2), G6PC3, GATA2, GFI1, HAX1, LAMTOR2(MAPBPIP), RAC2, SBDS, SLC37A4, TAZ, USB1(C16ORF57), VPS13B(COH1), VPS45, WAS, WIPF1

PDF Report

No

Day(s) Performed

Monday

Report Available

28 to 56 days

Specimen Retention Time

Extracted DNA: 2 months

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.

Test Definition: SCNGP

Congenital Neutropenia, Primary Immunodeficiency Disorder Panel (18 genes), Next-Generation Sequencing, Varies

CPT Code Information

81443

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
SCNGP	Congenital Neutropenia PID Panel	In Process

Result ID	Test Result Name	Result LOINC® Value
BA3910	Gene(s) Evaluated	48018-6
BA3911	Result Summary	50397-9
BA3912	Result Details	82939-0
BA3913	Interpretation	69047-9
BA3914	Additional Information	48767-8
BA3915	Method	85069-3
BA3916	Disclaimer	62364-5
BA3917	Reviewed by	18771-6