Congenital Neutropenia Panel

Versiti offers comprehensive genetic analysis to detect sequence variants and large deletions and duplications in 24 genes known to cause congenital neutropenia including cyclic neutropenia, non-syndromic neutropenia, and syndromic neutropenia with non-hematological manifestations. This panel can be ordered as:

- **Next Generation Sequencing (NGS) only;**
- **NGS with reflex to Array Comparative Genomic Hybridization (aCGH) Deletion/Duplication if sequencing does not identify clinically significant variants that fully explain the patient's phenotype;**
- **NGS with concurrent aCGH Deletion/Duplication (both testing methodologies performed simultaneously); or**
- **Deletion/Duplication by aCGH only.**

Congenital neutropenia is a heterogeneous group of disorders characterized by reduction in the absolute neutrophil count (ANC) of variable severity with or without extra-hematologic (syndromic) manifestations. When severe, it can be associated with recurrent severe infections, fever, and inflammation of the skin and mucous membranes. Some of the disorders in this group, in particular those that lead to severe congenital neutropenia (SCN), have a predisposition to myelodysplastic syndrome and acute myeloid leukemia (AML).

Diagnosis is based on clinical findings and serial measurement of the ANC showing persistently decreased neutrophil counts or variable neutrophil counts with severe neutropenia presenting with a predictable periodicity (cyclic neutropenia). The diagnosis of a specific congenital neutropenia disorder may be difficult to establish solely on functional studies or clinical history and many of the known causes of congenital neutropenia do not have a distinct clinical or laboratory phenotype. Accurate diagnosis provides information about phenotype and prognosis, guides medical management decisions, assists with the identification of affected family members, and allows for accurate genetic recurrence risk assessment.

Variants in several different genes are known to cause syndromic or non-syndromic congenital neutropenia, which may be inherited in an autosomal dominant, autosomal recessive or X-linked recessive manner. Some of the most common and more thoroughly characterized causes of congenital neutropenia are *ELANE*-related neutropenia (autosomal dominant inheritance) and *HAX1*-related neutropenia (autosomal recessive inheritance). Cyclic neutropenia is typically inherited in an autosomal dominant manner caused by heterozygous pathogenic variants in *ELANE*. Additional genes in this panel are associated with isolated neutropenia or congenital syndromes that have neutropenia as a common finding among other non-hematologic features.

The NGS panel evaluates for single nucleotide variants and small deletions and duplications, which are most commonly responsible for genetic disease. However, large deletions and duplications, also referred to as copy number variations (CNVs), are a known cause of genetic disorders, but can escape detection by next generation sequence analysis. Additional testing with aCGH Deletion/Duplication analysis is available for all genes on this panel to evaluate for large deletions and duplications encompassing one or more exons, or affecting an entire gene.

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Congenital Neutropenia Panel: gene, clinical phenotype and inheritance pattern.		
Gene	Clinical Phenotype	Inheritance
AP3B1	**Hermansky-Pudlak syndrome type 2 (HPS2):** oculocutaneous albinism of variable severity and mild bleeding due to a platelet storage pool disorder, as well as pulmonary fibrosis and neutropenia.	Autosomal Recessive
AP3D1	**Hermansky-Pudlak syndrome type 10 (HPS10):** oculocutaneous albinism of variable severity and mild bleeding due to a platelet storage pool disorder, as well as neutropenia, seizures and developmental delay.	Autosomal Recessive
CSF3R	**CSF3R-related congenital neutropenia (severe congenital neutropenia 7):** severe neutropenia that does not respond to granulocyte-colony stimulating factor (GCSF).	Autosomal Recessive
CXCR4	**WHIM syndrome:** Warts, Hypogammaglobulinemia, Infections and Myelokathexis.	Autosomal Dominant
ELANE	**ELANE-related neutropenia:** severe congenital neutropenia or cyclic neutropenia with an increased risk of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML).	Autosomal Dominant
G6PC3	G6PC3 deficiency (severe congenital neutropenia 4): severe neutropenia with or without syndromic hematologic and extra-hematologic associations.	Autosomal Recessive
GATA1	**GATA1-related X-linked cytopenia:** characterized by macrothrombocytopenia and/or anemia and neutropenia, with moderate bleeding due to platelet alpha granule deficiency.	X-linked Recessive
GATA2	**GATA1-related X-linked cytopenia:** characterized by macrothrombocytopenia and/or anemia and neutropenia, with moderate bleeding due to platelet alpha granule deficiency.	Autosomal Dominant
GFI1	**GFI1-related neutropenia (severe congenital neutropenia 2):** non-syndromic severe neutropenia.	Autosomal Dominant
HAX1	**HAX1-related neutropenia:** non-syndromic severe congenital neutropenia with an increased risk of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML).	Autosomal Recessive
JAGN1	**JAGN1-related neutropenia (severe congenital neutropenia 6):** severe congenital neutropenia with or without extra-hematologic manifestations.	Autosomal Recessive
LAMTOR2	**LAMTOR2-related neutropenia:** neutropenia, partial albinism, short stature and B and T cell deficiency.	Autosomal Recessive
LYST	**Chediak-Higashi syndrome:** partial oculocutaneous albinism, immunodeficiency, and a mild bleeding from platelet delta granule deficiency.	Autosomal Recessive
RAB27A	**Griscelli syndrome type 2:** pigmentary dilution (silver hair) , mild neutropenia and development of hemophagocytic lymphohistiocytosis due to uncontrolled T-lymphocyte expansion and macrophage activation syndrome.	Autosomal Recessive
RAC2	**RAC2-related neutrophil dysfunction (neutrophil immunodeficiency syndrome):** severe bacterial infections and impaired wound healing.	Autosomal Dominant
SBDS	**Shwachman-Diamond syndrome:** exocrine pancreatic dysfunction, bony metaphyseal dysostosis and varying degrees of marrow dysfunction with cytopenias (including neutropenia) and an increased risk of myelodysplastic syndrome and acute myeloid leukemia.	Autosomal Recessive
SLC37A4	**Glycogen storage disease type Ib:** growth retardation, hepatomegaly, renomegaly, hypoglycemia and other metabolic abnormalities, as well as neutropenia and neutrophil dysfunction.	Autosomal Recessive
TAZ	**Barth syndrome:** cardiomyopathy, short stature, neutropenia, hypocholesterolemia, impaired cognition, mild dysmorphic features and oxidative phosphorylation dysfunction .	X-linked
TCIRG1	**TCIRG1-related neutropenia:** non-syndromic neutropenia of variable severity	Autosomal Dominant
USB1	**Poikiloderma with neutropenia:** inflammatory eczematous rash followed by post-inflammatory poikiloderma, chronic neutropenia, sinopulmonary infections and bronchiectasis, nail dystrophy, palmar/plantar hyperkeratosis, short stature, hypogonadism, characteristic craniofacial features, non-healing skin ulcers, calcium deposits that form small nodules, and ulcers that do not heal; increased risk for MDS and rarely AML.	Autosomal Recessive
VPS13B	**Cohen syndrome:** variable developmental delay, microcephaly, distinctive facial features, hypotonia, joint hypermobility, retinopathy and neutropenia.	Autosomal Recessive
VPS45	**VPS45-related neutropenia (severe congenital neutropenia 5):** neutropenia with neutrophil dysfunction, bone marrow fibrosis and nephromegaly from renal extramedullary hematopoiesis.	Autosomal Recessive
WAS	**WAS-related disorders:** spectrum of disorders including Wiskott-Aldrich syndrome characterized by microthrombocytopenia, eczema and recurrent infections, X-linked thrombocytopenia and X-linked neutropenia.	X-linked
WIPF1	**Wiskott-Aldrich syndrome type 2 (WAS2):** recurrent infections, eczema, thrombocytopenia with normal platelet size, defective T cell proliferation and impaired natural killer cell function.	Autosomal Recessive

Indications for testing:

Congenital Neutropenia Panel (NGS and/or aCGH), order code 4845:

The Congenital Neutropenia Panel should be considered:

- In patients with chronic/lifelong neutropenia presenting with or without extra-hematologic manifestations
- In patients in whom a family history of congenital neutropenia is reported but unspecified, without an affected relative available for confirmation

Single Gene Analysis (order code 4855) or Custom Blood Disorder Panel (Order Code 4850), (NGS and/or aCGH):

Analysis of genes included in this panel may also be ordered as a standalone Single Gene Analysis or as a Custom Blood Disorder Panel (2-10 genes), by NGS and/or by aCGH, as dictated by the patient's clinical and laboratory phenotype, as well as their ancestry, or to supplement previous genetic testing.

Targeted Familial Variant Analysis (order code 4970):

Targeted variant analysis for clinical diagnosis, carrier identification, or prenatal diagnosis can also be performed on any gene in the panel when the pathogenic variant(s) is known in the family. If the proband was not tested at Versiti, a control sample may be needed (please call the laboratory to discuss). If the familial variant is a large deletion or duplication, aCGH for the involved gene is required.

For clinical questions about laboratory tests and test utilization support, contact Versiti Client Services: (414) 937-6396 or 800-245-3117, Option 1, to be directed to our genetic counselors and clinical support team.

Informed Consent

It is recommended that healthcare providers obtain informed consent from the patient when genetic testing is ordered, consistent with any applicable state laws and regulations, documenting that the patient has been advised of understands the indications for and implications of the genetic test. This panel is designed for clinical detection of germline genetic variants in genes with strong or definitive evidence for causality of neutropenia or neutrophil dysfunction. Test results may nonetheless yield genetic findings that may be unrelated to the current clinical presentation, and/or may carry individual or familial implications such as risk for syndromic manifestation, predisposition to malignancy, and/or reproductive implications (such as carrier status). If needed, an informed consent form for Versiti Hematology Genetics testing can be found at http://www.versiti.org/hg under *forms*.

Test method:

NGS: This next-generation sequencing assay analyzes the complete coding region of 24 genes plus a minimum 30bp of non-coding DNA, including intron-exon boundaries, and is compared to the build GRCh37.p13 reference sequence. These targeted regions are captured by hybridization, amplified, and sequenced by massively parallel sequencing. Regions will have a minimum coverage of 50x and those regions with less than 50 sequencing reads or low quality coverage are supplemented with Sanger sequencing. All regions are covered by bidirectional analysis. Variants are identified by a customized bioinformatics pipeline, analyzed and comprehensively interpreted by our team of practicing hematologists with expertise in non-malignant hematology and laboratory diagnostics, scientists, and genetic counselors. All reported variants, including pathogenic, likely pathogenic, and variants of uncertain significance, are confirmed by Sanger sequencing. For prenatal testing, analysis of variable number tandem repeats (VNTR) is used to confirm results are not affected by maternal cell contamination

aCGH: The specific genes are analyzed for copy number variations due to deletion or duplication by high density gene-focused array Comparative Genomic Hybridization. Probes are approximately 60bp in length and density of coverage in exonic regions is a minimum of 4 probes per 500 bp. Genomic DNA for the samples and gender-matched references are denatured, labeled with fluorescent dye and hybridized, the array is washed and scanned, and analysis is performed for the specific genes requested.

Assay sensitivity and limitations:

NGS: The analytical sensitivity of the NGS test is >99% for single nucleotide changes and insertions and deletions of less than 20 bp. NGS analysis is not designed to detect large deletions or duplications (>20 bp), or variants that are outside the regions sequenced. Low level mosaicism will not be detected by this sequencing methodology.

aCGH: Chromosomal rearrangements (i.e., translocations, inversions) or point mutations that may be the cause of the clinical phenotype cannot be detected via aCGH. Any exonic deletion or duplication smaller than 500bp may not be detected. Low level of mosaicism will not be detected by aCGH. Probe performance could be affected by multiple SNPs in a given region. Breakpoints occurring outside the targeted gene(s) will not be defined.

Clinical Sensitivity

The clinical sensitivity of comprehensive genetic testing (NGS and aCGH) of the 24 genes known to be associated with congenital neutropenia is highest in patients presenting with lifelong neutropenia or a clear cyclic pattern.

Reporting of results:

Results are classified and reported in accordance with ACMG next-generation sequencing and copy number variation standards. Sequence variants and large deletions and duplications predicted to be pathogenic, likely pathogenic, and of uncertain significance will be reported; variants classified as likely benign or benign are typically not reported but such data are available upon request. Sequence variants are described using standard Human Genome Variation Society (HGVS) nomenclature (http://hgvs.org); copy number variants are described in accordance with the International System for Human Cytogenomic Nomenclature (ISCN).

Specimen Requirements

Parental/Patient/Pediatric: 3-5 mL Whole blood (EDTA tube, lavender top), 2-5 mL Bone marrow (EDTA tube, lavender top), 3-4 Buccal swabs, or ≥1ug of DNA at ≥50ng/uL of High Quality DNA.

Fetal: 7-15 mL amniotic fluid, 5-10 mg chorionic villi; back up culture of amniocytes or chorionic villi is highly recommended. Cultured: Two T25 flasks cultured amniocytes or chorionic villi ($2x10^6$ minimum). Maternal blood sample of 3-5 mL Whole blood (EDTA tube, lavender top) is requested for all prenatal samples for maternal cell contamination studies. For questions please contact the laboratory to discuss sample requirements.

SHIP

Shipping Requirements

Ship on an ice pack or at room temperature. Protect from freezing. Place the specimen and the requisition into plastic bags and seal. Insert into a Styrofoam container, seal and place into a sturdy cardboard box, and tape securely. Ship the package in compliance with your overnight carrier guidelines. Label with the following address:

Client Services/Diagnostic Laboratory
Versiti
638 N. 18th St
Milwaukee, WI, 53233

ORDER

Required Forms

Please complete all pages of the requisition form. Clinical history (including patient's ethnicity, clinical diagnosis, family history, and relevant laboratory findings) is necessary for optimal interpretation of genetic test results and recommendations. Clinical and laboratory history can either be recorded on the requisition form or clinical and laboratory reports can be submitted with the sample.

CPT Codes/Billing/Turnaround Time

Test code: 4845

CPT Codes: For recommended CPT codes, visit the Versiti.org test menu.

Turnaround time: 21 days

For additional information related to shipping, billing or pricing, please contact Versiti Client Services: (414) 937-6396 or 800-245-3117, Option 1, or LabInfo@versiti.org

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Variant interpretation references

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13. Results are classified and reported in accordance with ACMG next-generation sequencing standards. Variants predicted to be pathogenic, likely pathogenic, and of uncertain significance will be reported; variants classified as likely benign or benign are typically not reported but such data are available upon request.
14. Sequence variants are described using standard Human Genome Variation Society (HGVS) nomenclature (http://hgvs.org).

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