Inherited Neutropenia Gene Sequencing Panel

Genes Tested:

AK2	*AP3B1*	*CD40LG*	*CLPB*
CSF3R	*CXCR2*	*CXCR4*	*DNAJC21*
EFL1	*EIF2AK3*	*ELANE*	*G6PC3*
GATA1	*GATA2*	*GFI1*	*HAX1*
HYOU1	*JAGN1*	*LAMTOR2*	*LYST*
MRTFA (MKL1)	*RAB27A*	*RAC2*	*RMRP*
RUNX1	*SBDS*	*SLC37A4*	*SMARCD2*
SRP54	*STK4*	*TAZ*	*TCIRG1*
TCN2	*TP53*	*USB1*	*VPS13B*
VPS45	*WAS*	*WDR1*	*WIPF1*

Description:

This panel detects the most common genetic causes of severe congenital neutropenia as well as genetic syndromes often associated with neutropenia, including Barth syndrome, Chediak-Higashi syndrome, Clericuzio-type poikiloderma with neutropenia, Cohen syndrome, *GATA1*-related X linked cytopenia, *GATA2* deficiency, glycogen storage disease type 1B, Griscelli syndrome type 2, Hermansky-Pudlak syndrome type 2, p14 deficiency, Shwachman-Diamond syndrome, WHIM syndrome, and Wiscott-Aldrich syndrome. Variants in *RAC2* have been reported in association with neutrophil immunodeficiency syndrome.

Severe congenital neutropenia (SCN) is a disorder of neutrophil production. The incidence of SCN is approximately 3-4 per million births. Children with SCN typically present with severe neutropenia, fever, and recurrent infections of the upper respiratory tract, lungs and skin within the first year of life. Mutations in *ELANE (ELA2)* are the most common cause of SCN, as well as of cyclic neutropenia. *ELANE* encodes neutrophil elastase, which targets bacterial virulence proteins and serves as the cell's first line of defense against overwhelming bacterial infection. Nonsense and frameshift variants affecting the carboxyl terminus are quite common, while missense variants are seen more commonly in cyclic neutropenia patients. However, there is considerable overlap of genotype with phenotype, even within families. Mutations in *GFI1, HAX1, G6PC3, VPS45* and *CFS3R* are much less frequent causes of severe congenital neutropenia. Variants within the Cdc42 binding site of *WAS* are also associated with an X-linked form of congenital neutropenia.

The diagnostic criteria for SCN include:

- Early childhood onset of profound neutropenia ($<0.5 \times 10^9$/L)
- Recurrent life-threatening bacterial infections
- Promyelocytic maturation arrest in the bone marrow

Syndromic neutropenia: Significant neutropenia is a common feature of several genetic syndromes associated with extra-hematopoietic abnormalities including Barth syndrome, Cohen syndrome secondary to *VPS13B* variants, Chediak-Higashi syndrome, Clericuzio-type poikiloderma with neutropenia, *GATA1*-related X linked cytopenia, *GATA2* deficiency, glycogen storage disease type 1B, Griscelli syndrome type 2, Hermansky-Pudlak syndrome type 2, p14 deficiency, Shwachman-Diamond syndrome and WHIM syndrome.

This panel also includes sequencing for somatic level variants in *CSF3R, RUNX1,* and *TP53.* Acquired variants in *CSF3R* have been reported in patients with severe congenital neutropenia, as well as in patients whose SCN has undergone progression to myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) (Germeshausen et al. 2007; Touw 2015). Acquired variants in *RUNX1* have been reported in patients with MDS/AML who have undergone progression from SCN, including in combination with previously acquired *CSF3R* variants (Skokowa et al. 2014). Acquired variants in *TP53* have been reported in patients with Shwachman-Diamond

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Genetic Conditions Commonly Associated with Neutropenia

Gene	Inheritance	Condition
AK2	AR	Reticular dysgenesis
AP3B1	AR	Hermansky-Pudlak type 2
CD40LG	X linked	X-linked hyper IgM syndrome
CLPB	AR	3-methylglutaconic aciduria type VII, with cataracts, neurologic involvement and neutropenia
CSF3R	AD, AR and somatic	SCN7 (germline); predisposition to myelodysplastic syndrome (somatic)
CXCR2	AR	Myelokathexis
CXCR4	AD	WHIM syndrome
DNAJC21	AR	Familial bone marrow failure syndrome type 3
EIF2AK3	AR	Wolcott-Rallison syndrome
EFL1	AR	Schwachman Diamond syndrome 2
ELANE (ELA2)	AD	SCN1
G6PC3	AR	SCN4, nonsyndromic SCN, Dursun syndrome
GATA1	X linked	GATA1-related X-linked cytopenia
GATA2	AD	GATA2 deficiency
GFI1	AD	SCN2
HAX1	AR	SCN3, Kostmann syndrome
HYOU1	AR	Immunodeficiency and hypoglycemia
JAGN1	AR	SCN6
LAMTOR2 (ROBLD3)	AR	p14 deficiency
LYST	AR	Chediak-Higashi syndrome
MRTFA (MKL1)	AR	Neutropenia with combined immune deficiency
RAB27A	AR	Griscelli syndrome type 2
RAC2	AR	Neutrophil immunodeficiency syndrome
RMRP	AR	Cartilage-hair hypoplasia
RUNX1	AD and somatic	Platelet disorder (germline), predisposition to myelodysplastic syndrome / acute myeloid leukemia (somatic)
SBDS	AR	Shwachman Diamond syndrome (SDS)
SLC37A4	AR	Glycogen storage disease type IB
SMARCD2	AR	Specific granule deficiency 2
STK4	AR	STK4 deficiency
TAZ	X linked	Barth syndrome
TCIRG1	AR, AD	Osteopetrosis (AR), Congenital neutropenia (AD)
TCN2	AR	Transcobalamin II deficiency
TP53	AD and somatic	Li Fraumeni syndrome or BMF5 (germline), transformation to myelodysplastic syndrome/ acute myeloid leukemia in patients with Schwachman Diamond syndrome (somatic)
USB1	AR	Clericuzio-type poikiloderma with neutropenia
VPS13B	AR	Cohen syndrome; congenital neutropenia with retinopathy
VPS45	AR	SCN5
WAS	X linked	Wiskott-Aldrich syndrome, X-linked
WDR1	AR	WDR1 deficiency
WIPF1	AR	Wiskott-Aldrich syndrome

syndrome (SDS), and may be an early event predisposing SDS patients to transformation to MDS/AML (Xia et al. 2018). Variants in these 3 genes are reported if the variant allele frequency is 5% or higher.

Indications:

Inherited Neutropenia Panel by NGS:

- Confirmation of genetic diagnosis in a patient with a clinical diagnosis of primary neutropenia or associated syndrome
- Carrier identification in individuals with a family history of inherited neutropenia of unknown genetic basis.

Gene Specific Sequencing:

- Confirmation of genetic diagnosis in a patient with neutropenia and in whom ancillary testing or clinical history suggests a specific genetic diagnosis.

Variant Specific Analysis:

- Presymptomatic testing of at-risk family members for medical management
- Carrier testing of family members for recurrence risk assessment
- Prenatal diagnosis of an at-risk fetus, after confirmation of variant(s) in the parent(s) and by prior arrangement only.

Specimen:

At least 3 mLs whole blood in a lavender top (EDTA) tube or saliva in an Oragene saliva kit. Please call 513-636-4474 for a free saliva collection kit.

Note: For post-transplant patients, we accept pre-transplant samples or post-transplant skin fibroblasts ONLY (blood, saliva, and cytobrushes are not accepted). Culturing of skin fibroblasts is done at an additional charge.

Testing Methodology:

Inherited Neutropenia Panel by NGS: This test is performed by enrichment of the coding exons, flanking intronic and untranslated regions (5' and 3'), as well as known pathogenic variants (HGMD 2018.4) in the promoter and deep intronic regions of the genes specified above using oligonucleotide probe hybridization followed by next-generation sequencing with >50X coverage at every target base. Regions with <50X will be filled in by Sanger sequencing. All pathogenic and likely pathogenic variants, as well as variants of unknown (indeterminate) significance, as determined bioinformatically, are confirmed by Sanger sequencing. The limit of detection of somatic variants in *CSF3R, RUNX1,* and *TP53* with this methodology is 5%. Somatic variants with <20% variant allele frequency may not be confirmed by Sanger sequencing. A detailed non-coding variant list is available upon request.

Test Sensitivity:

Analytical Sensitivity: The sensitivity of DNA sequencing is over 99% for the detection of nucleotide base changes, small deletions and insertions in the regions analyzed. Somatic variants in *TP53, RUNX1,* and *CSF3R* are expected to be identifiable when they are present at a variant allele frequency greater than 5%.

Limitations: Variants in regulatory regions and non-reported variants in untranslated regions may not be detected by this test. Large deletions/ duplications, large insertions and other complex genetic events will not be identified using sequencing methodology.

Turn-Around Time:

- Inherited Neutropenia NGS panel: up to 6 weeks
- Single gene sequencing: 28 days

CPT Codes:

- **Inherited Neutropenia NGS Panel:** 81443
- **Single gene sequencing, targeted variant analysis, and deletion/duplication:** call for information.

Please call 1-866-450-4198 for current pricing, insurance preauthorization or with any billing questions.

Shipping Instructions:

Please enclose **test requisition** with sample.
All information must be completed before sample can be processed.

Place samples in styrofoam mailer and ship at room temperature by overnight Federal Express to arrive Monday through Friday.

Ship to:

Cytogenetics and Molecular Genetics Laboratories
3333 Burnet Avenue NRB 1042
Cincinnati, OH 45229
513-636-4474

Results:

Results will be reported to the referring physician or health care provider as specified on the requisition form.

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