Inherited Neutropenia Panel by next-generation sequencing (NGS)

Genes Tested

AP3B1	*CSF3R*	*CXCR4*	*ELANE (ELA2)*
G6PC3	*GATA1*	*GATA2*	*GFI1*
HAX1	*LAMTOR2 (ROBLD3)*	*LYST*	*RAB27A*
RAC2	*SBDS*	*SLC37A4*	*TAZ*
USB1	*VPS13B*	*VPS45*	*WAS*
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. Biallelic mutations in *RAC2* have been reported in association with neutrophil immunodeficiency syndrome. Germline mutation in *CSF3R* have been reported in patients with severe congenital neutropenia while acquired (somatic) mutations in *CSF3R* are a risk factor for malignant transformation in patients with inherited neutropenia and may be detected by this test, if the mutation load exceeds 20% in the tissue analyzed.

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 mutations affecting the carboxyl terminus are quite common, while missense mutations are seen more commonly in cyclic neutropenia patients. However, there is considerable overlap of genotype with phenotype, even within families. Mutations in *GFI1, HAX1, G6PC, VPS45* and *CFS3R* are much less frequent causes of severe congenital neutropenia. Mutations 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

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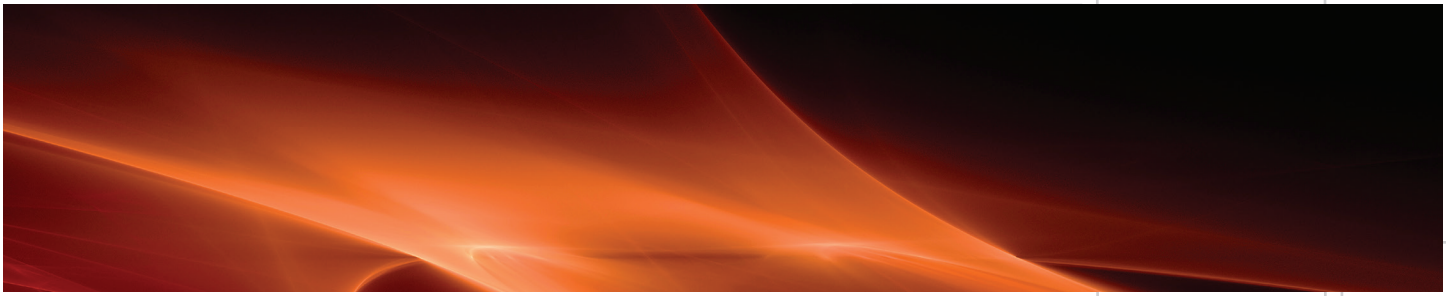

Syndromic neutropenia: Significant neutropenia is a common feature of several genetic syndromes associated with extra-hematopoietic abnormalities including Barth syndrome (neutropenia, often intermittant in 90%), Cohen syndrome secondary to *VPS13B* mutations (intermittent neutropenia in 92%), Chediak-Higashi syndrome (neutropenia in >90%), Clericuzio-type poikiloderma with neutropenia (chronic neutropenia in >50%), GATA1-related X linked cytopenia (variable neutropenia), GATA2 deficiency (mild chronic neutropenia in 47%), glycogen storage disease type 1B (87% develop intermittent or chronic neutropenia), Griscelli syndrome type 2 (mild neutropenia), Hermansky-Pudlak syndrome type 2 (chronic severe neutropenia), p14 deficiency, Shwachman-Diamond syndrome (chronic or intermittent neutropenia in 98%) and WHIM syndrome (chronic neutropenia in 100%).

Malignant transformation, i.e. myelodysplasia and acute myelogenous leukemia (AML), is a significant risk in patients with SCN and SBDS. A recent study suggests that the risk of malignant transformation is greatly increased in the presence of acquired truncating mutations in *CSF3R* in patients with SCN [Skokowa et al 2014].

Genetic Conditions Commonly Associated with Neutropenia

Gene	Inheritance	Condition
AP3B1	AR	Hermansky-Pudlak type 2
CSF3R	AD, AR and somatic	Severe congenital neutropenia (germline); predisposition to MDS (somatic)
CXCR4	AD	WHIM syndrome
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
LAMTOR2 (ROBLD3)	AR	p14 deficiency
LYST	AR	Chediak-Higashi syndrome
RAB27A	AR	Griscelli syndrome type 2
RAC2	AR	Neutrophil immunodeficiency syndrome
SBDS	AR	Shwachman-Diamond syndrome (SDS)
SLC37A4	AR	Glycogen storage disease type IB
TAZ	X linked	Barth syndrome
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 thrombocytopenia, X-linked congenital neutropenia
WIPF1	AR	Wiskott-Aldrich syndrome 2

These conditions are summarized in the Clinician's Guide.

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.

Mutation Specific Analysis:

- Presymptomatic testing of at-risk siblings for medical management and prior to bone marrow donation
- Carrier identification in individuals in whom specific mutation(s) have been identified in the family member with neutropenia
- Prenatal diagnosis of an at-risk fetus, after carrier testing of parent(s) and by prior arrangement only.

Specimen:

Inherited Neutropenia Panel by NGS: At least 5 mLs whole blood in a lavender top (EDTA) tube.

Gene Specific Sequencing or Mutation Specific Analysis: At least 3 mLs whole blood in a lavender top (EDTA) tube.

Note: Saliva samples are required for analysis in patients who have undergone bone marrow transplantation and may facilitate DNA isolation in patients undergoing chemotherapy or in individuals with leukopenia. Please call 513-636-4474 for a free saliva collection kit.

Testing Methodology:

Next Generation Sequencing Panel: This test is performed by enrichment of the exons, flanking intronic and un-translated regions (5' and 3') of the genes specified above using microdroplet PCR technology followed by next-generation sequencing with >20 fold coverage at every target base. All pathogenic and novel variants, as well as variants of unknown (indeterminate) significance, as determined bioinformatically, are confirmed by Sanger sequencing.

Single Gene Sequencing/Mutation Specific Analysis: Sanger sequencing following PCR amplification of the specified coding and exon/intron boundaries of the specified gene. Sanger sequencing is available for every gene in the panel.

Sensitivity:

Clinical Sensitivity: At least 70-80% of the genetic causes of congenital or inherited neutropenia are identified by this panel. Approximately 60% of North American patients with severe congenital neutropenia have a mutation in *ELANE,* as do approximately 90% of patients with cyclic neutropenia. Mutations in *HAX1, G6PC3, GFI1, WAS* and *CSF3R* account for 10-20% of patients collectively. Patients with genetic syndromes that include neutropenia as a common finding, as well as patients in whom the genetic basis of neutropenia has not been identified account for the remaining 20-30%.

Over 90% of patients with Barth syndrome have mutations in *TAZ;* approximately 2/3 of which can be detected by this test. Only 35% of patients with Cohen syndrome have mutations in *VPS13B,* but nearly all patients who have Cohen syndrome with neutropenia have identifiable mutation. Approximately 90% of patients with a clinical diagnosis of Chediak-Higashi syndrome or Shwachman-Diamond syndrome will have mutations that are identifiable by this test. Approximately 80% of patients with GATA2 deficiency will have identifiable mutations. The clinical sensitivity for Clericuzio-Type poikiloderma with neutropenia, GATA1-related X linked cytopenia, glycogen storage disease type 1B, neutrophil immunodeficiency syndrome, p14 deficiency, WHIM syndrome and WAS2 has not been determined due to the rarity of these conditions.

Deletion/duplication analysis may be indicated as a follow-up test in symptomatic patients with a normal NGS sequencing result or a single (heterozygous) mutation in association with an autosomal recessively inherited disorder, and is clinically available at an additional charge.

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 mutations in *CSF3R* are likely to be detected when they are present in >20% of cells analyzed. Large deletions, regulatory mutations and complex rearrangements are not detected by this test and have been reported in many of the genes on this panel.

Limitations: Mutations in regulatory regions or other untranslated regions are not detected by this test. Large deletions involving entire single exons or multiple exons, large insertions and other complex genetic events have been reported in many of these genes and will not be identified using this test methodology. Rare primer site variants may lead to erroneous results.

Turn-Around Time:

- **Inherited Neutropenia Panel:** 12 weeks
- **Single Gene Sequencing:** 4 weeks

CPT Codes:

• Inherited Neutropenia NGS Panel:	81479x17, 81406x3, 81408
• *SLC37A4, TAZ* and *WAS*:	81406
• All other genes in panel:	81479
• Family specific study:	81403

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

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

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

References:

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