

Erythrocytosis Gene Sequencing Panel

Genes Tested:

BHLHE41, BPGM, CALR, CYB5R3, EGLN1 (PHD2), EGLN2 (PHD1), EGLN3 (PHD3), EPAS1 (HIF2A), EPO, EPOR, GFI1B, HBA1, HBA2, HBB, HIF1A, HIF1AN (FIH), HIF3A, JAK2, KDM6A, MPL, OS9, PIEZO1, PKLR, SH2B3, SLC30A10, VHL, ZNF197

Description:

Erythrocytosis is defined as increased red blood cell mass, indicated by increased hemoglobin and hematocrit levels, and is caused by dysregulation in red blood cell production. Erythropoietin (Epo) levels may be low or high, depending on the underlying cause. Erythrocytosis presents with variable clinical features such as plethora, headache, dizziness, fatigue, visual and auditory disturbance, altered mental status, and/or arterial or venous thromboembolic events. Erythrocytosis can be acquired or congenital. Acquired cases may be secondary to chronic tissue hypoxia or primary due to bone marrow disease, such as polycythemia vera caused by somatic (clonal) pathogenic variants in the *JAK2* gene (most commonly *JAK2* V617F).

Congenital erythrocytosis is also classified as primary or secondary. Primary congenital erythrocytosis is due to pathogenic variants in the *EPOR* and *JAK2* genes, increasing sensitivity to erythropoietin. Secondary congenital erythrocytosis can be due to pathogenic variants in genes of the hypoxia-sensing pathway increasing erythropoietin production (*EGLN1*, *EPAS1*, and *VHL*) or due to high oxygen affinity of hemoglobin leading to tissue hypoxia. High oxygen affinity of hemoglobin can be due to decreased 2,3-BPG caused by *BPGM* variants, or due to certain missense variants in *HBA1*, *HBA2*, and *HBB*, which lead to increased oxygen affinity of the hemoglobin tetramer.

The inheritance of primary and familial erythrocytosis can be autosomal dominant or autosomal recessive. The genes on the Erythrocytosis Gene Sequencing Panel are grouped into two categories: Tier 1, and Tier 2, based on the current evidence of human disease association. Tier 1 contains 17

genes known to be associated with Erythrocytosis or diseases with polycythemia phenotype. Tier 2 consists of 10 genes that show limited evidence in erythrocytosis or are the genes in or related to the oxygen-sensing (hypoxia-inducing factor, HIF) pathway, involved in the pathogenesis of erythrocytosis. The genes in Tier 2 may be upgraded to Tier 1 as additional knowledge emerges. The Erythrocytosis Gene Sequencing panel uses a combination of Exome slice based on Twist Human Comprehensive Exome and Sanger sequencing to detect variants in these 27 genes. We use Sanger sequencing to detect variants in the *HBA1*, *HBA2*, and *HBB* genes.

Indications:

Erythrocytosis Gene Sequencing Panel:

- Confirmation of genetic diagnosis in a patient with a clinical diagnosis of polycythemia vera or congenital erythrocytosis

Gene Specific Sequencing:

- Confirmation of genetic diagnosis in a patient with erythrocytosis and in whom a specific gene is suspected

Variant Specific Analysis:

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

Genetic Conditions Commonly Associated with Erythrocytosis

Tier 1 Genes

Gene	Inheritance	Conditions
<i>BPGM</i>	AR	Erythrocytosis, familial, 8
<i>CALR</i>	somatic	Erythrocytosis, somatic; Myelofibrosis, somatic; Thrombocytosis, somatic
<i>CYB5R3</i>	AR	Methemoglobinemia, type I/II
<i>EGLN1 (PHD2)</i>	AD	Erythrocytosis, familial, 3
<i>EPAS1 (HIF2A)</i>	AD	Erythrocytosis, familial, 4
<i>EPO</i>	AD	Erythrocytosis, familial, 5
<i>EPOR</i>	AD	Erythrocytosis, familial, 1
<i>HBA1</i>	AD	Erythrocytosis due to hemoglobin with high oxygen affinity
<i>HBA2</i>	AD	Erythrocytosis due to hemoglobin with high oxygen affinity
<i>HBB</i>	AD	Erythrocytosis due to hemoglobin with high oxygen affinity
<i>JAK2</i>	AD, somatic	Erythrocytosis; Polycythemia vera, somatic
<i>MPL</i>	AD, somatic	Myelofibrosis, somatic; Thrombocytosis, AD/ somatic
<i>PIEZO1</i>	AD	Dehydrated hereditary stomatocytosis with or without pseudohyperkalemia and/or perinatal edema*
<i>PKLR</i>	AD	Adenosine triphosphate, elevated, of erythrocytes
<i>SH2B3</i>	AD, somatic	Erythrocytosis, somatic
<i>SLC30A10</i>	AR	Hypermanganesemia with dystonia 1
<i>VHL</i>	AR	Erythrocytosis, familial

*Evidence showed increased incidence of germline *PIEZO1* mutations in individuals with idiopathic erythrocytosis (PMID: 33181827) and mild erythrocytosis as a presenting manifestation of *PIEZO1* associated erythrocyte volume disorders (PMID: 31298594).

Tier 2 Genes

Gene	Inheritance	Conditions/Pathways
<i>BHLHE41</i>	likely AR	related to the HIF pathway and identified in WGS500 project
<i>EGLN2 (PHD1)</i>	likely AD	Oxygen-sensing pathway and key gene in the HIF pathway
<i>EGLN3 (PHD3)</i>	likely AD	Oxygen-sensing pathway and key gene in the HIF pathway
<i>GFI1B</i>	likely AR	Erythropoiesis and identified in WGS500 project
<i>HIF1A</i>	likely AD	Oxygen-sensing pathway and key gene in the HIF pathway
<i>HIF1AN (FIH)</i>	unknown	Oxygen-sensing pathway and key gene in the HIF pathway
<i>HIF3A</i>	likely AD	Oxygen-sensing pathway and key gene in the HIF pathway
<i>KDM6A</i>	X linked	Oxygen-regulated demethylase and identified in WGS500 project
<i>OS9</i>	unknown	related to the HIF pathway
<i>ZNF197</i>	unknown	related to the HIF pathway

Key: AD= Autosomal dominant; AR= Autosomal Recessive

What Is Reported?

Variants that will be discussed in detail in the report:

- Pathogenic and likely pathogenic variants
- Variants of uncertain clinical significance may be discussed in detail on a case-by-case situation

Variants that will be listed in the report:

- Variants of uncertain clinical significance
- Variants that predict an increased risk of diseases, but do not cause a disease by themselves (risk alleles)

What is not reported:

- Variants in genes not included in the predefined gene list
- Variants classified as benign or likely benign

Note: Erythrocytosis Gene Sequencing Panel cases with negative or uncertain findings can be reflexed to Whole Exome Sequencing (WES). A separate test order and a signed consent form is required for all WES testing. In addition, including biological parental samples is strongly encouraged to assist with the analysis of WES and to increase test yield. Reflex to WES orders can either be placed simultaneously or separately. Separate reflex to WES orders are subject to review prior to the initiation of testing. Please see our website at www.cincinnatichildrens.org/exome to obtain a WES test requisition and consent form.

Methodology:

Erythrocytosis gene sequencing panel uses Human Comprehensive Exome kit from Twist Bioscience to capture the exonic regions of genes from the genomic DNA extracted from the patient. Targeted regions are sequenced using an Illumina sequencing system with paired-end reads. Sequence reads are aligned to the human reference genome (build UCSC hg19). Variants within exons and flanking sequences are identified and evaluated by a validated in-house developed bioinformatics analysis pipeline that includes the usage of GATK and Fabric Genomic Analysis platform. Data quality is assessed to confirm it has a minimum coverage of 20X for >95% of targets of interest. *HBA1*, *HBA2*, *HBB* have gene regions with homology which may generate suboptimal NGS data with potential false negative results. Therefore, these three genes are analyzed by Sanger sequencing.

Technical Limitations:

- Pathogenic variants may be present in a portion of the genes not covered by this test or in regions with suboptimal data due to homologous issue, poly-nucleotides, or nucleotide repeats, and therefore may not be identified. Thus, the absence of identified pathogenic variants does not exclude the possibility of a genetic etiology for the patient's symptoms.
- Certain types of mutations are not detected. Only single base pair changes or small insertions or deletions of DNA are detected. Large deletions, duplications, or rearrangements, mitochondrial genome mutations, repeat expansions, low level mosaicism and many epigenetic defects may not be detected.

Low coverage (<20X) regions

GENE	REGION	LENGTH	CHROM	EXON_START	EXON_END
<i>BHLHE41</i>	NM_030762:c.938_945	8 bps	12	26275503	26275510
<i>CYB5R3</i>	NM_000398:c.-8_11	19 bps	22	43045315	43045329
<i>HIF3A</i>	NM_152796:c.1274-8_*8	96 bps	19	46841476	46841571
<i>KDM6A</i>	NM_021140:c.1330-8_1330-6	3 bps	X	44920561	44920563
<i>KDM6A</i>	NM_021140:c.1527+7_1527+8	2 bps	X	44922000	44922001

Please note: These regions represent the low coverage (<20X) regions identified during our test validation. For specific patient cases, these regions may vary.

Turn-Around Time:

42 days (6 weeks)

Specimen:

At least 3 mls whole blood in a lavender top (EDTA) tube or saliva in an Oragene saliva kit. Please call the lab at 513-636-4474 for a free saliva collection kit. Label the tube with the patient's name, birth date, and date of collection. Alternatively, 10 mcg of DNA extracted by a CLIA certified lab may be submitted.

CPT Codes:

- **Erythrocytosis Gene Sequencing Panel:** 81404, 81479(x3)
- **Single Gene Testing and Targeted Variant Analysis:** call for information
- **Deletion/Duplication analysis:** call for information

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 requisition form.

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 Saturday.

Ship to:

Genetics and Genomics Diagnostic Laboratory
3333 Burnet Avenue NRB 1042
Cincinnati, OH 45229
513-636-4474

References:

Bento, C., et al. (2014). Genetic basis of congenital erythrocytosis: mutation update and online databases. *Human mutation*, 35(1), 15–26.

Bento, C., McMullin, M. F., Percy, M., & Cario, H. (2016). Primary Familial and Congenital Polycythemia. In M. P. Adam (Eds.) et al., *GeneReviews*®. University of Washington, Seattle.

Broséus, J., et al. (2014). Presence of calreticulin mutations in JAK2-negative polycythemia vera. *Blood*, 124(26), 3964–3966.

Cario, H., et al. (2013). Erythrocytosis in children and adolescents—classification, characterization, and consensus recommendations for the diagnostic approach. *Pediatric blood & cancer*, 60(11), 1734–1738.

Camps, C., et al. (2016). Gene panel sequencing improves the diagnostic work-up of patients with idiopathic erythrocytosis and identifies new mutations. *Haematologica*, 101(11), 1306–1318.

Filser, M., et al. (2021). Increased incidence of germline *PIEZO1* mutations in individuals with idiopathic erythrocytosis. *Blood*, 137(13), 1828–1832.

Gesang, L., et al. (2019). Whole-Genome Sequencing Identifies the Egl Nine Homologue 3 (*egln3/phd3*) and Protein Phosphatase 1 Regulatory Inhibitor Subunit 2 (*PPP1R2P1*) Associated with High-Altitude Polycythemia in Tibetans at High Altitude. *Disease markers*, 2019, 5946461.

Hong, W. J., & Gotlib, J. (2014). Hereditary erythrocytosis, thrombocytosis and neutrophilia. *Best practice & research. Clinical haematology*, 27(2), 95–106.

Kapralova, K., et al. (2016). Cooperation of germ line *JAK2* mutations E846D and R1063H in hereditary erythrocytosis with megakaryocytic atypia. *Blood*, 128(10), 1418–1423

Kristan, A., Debeljak, N., & Kunej, T. (2019). Genetic variability of hypoxia-inducible factor alpha (*HIF1A*) genes in familial erythrocytosis: Analysis of the literature and genome databases. *European journal of haematology*, 103(4), 287–299.

Knight, T., et al. (2019). Mild erythrocytosis as a presenting manifestation of *PIEZO1* associated erythrocyte volume disorders. *Pediatric hematology and oncology*, 36(5), 317–326.

Lasho, T. L., et al. (2010). *LNK* mutations in *JAK2* mutation-negative erythrocytosis. *The New England journal of medicine*, 363(12), 1189–1190.

Maslah, N., et al. (2017). The role of *LNK/SH2B3* genetic alterations in myeloproliferative neoplasms and other hematological disorders. *Leukemia*, 31(8), 1661–1670.

McMullin M. F. (2016). Congenital erythrocytosis. *International journal of laboratory hematology*, 38 Suppl 1, 59–65.

Milosevic Feenstra, et al. (2016). Whole-exome sequencing identifies novel *MPL* and *JAK2* mutations in triple-negative myeloproliferative neoplasms. *Blood*, 127(3), 325–332.

Oh, S. T., et al. (2010). Novel mutations in the inhibitory adaptor protein *LNK* drive *JAK-STAT* signaling in patients with myeloproliferative neoplasms. *Blood*, 116(6), 988–992.

Spolverini, A., et al. (2013). Infrequent occurrence of mutations in the PH domain of *LNK* in patients with *JAK2* mutation-negative ‘idiopathic’ erythrocytosis. *Haematologica*, 98(9), e101–e102.