Erythrocytosis Gene Sequencing Panel

Condition	Gene(s)	Inheritance*
Erythrocytosis, familial Polycythemia vera, somatic	*JAK2*	AD with variable penetrance, somatic
Primary congenital erythrocytosis Primary familial congenital polycythemia (PFCP) ECYT1 (133100)	*EPOR*	AD
Erythrocytosis due to bisphosphoglycerate mutase deficiency causing high oxygen affinity to hemoglobin	*BPGM*	AR
Erythrocytosis, familial ECYT3 (609820) ECYT4 (611783)	*EGLN1* *EPAS1*	AD
Erythrocytosis, familial Chuvash polycythemia ECYT2 (263400)	*VHL*	AR
Erythrocytosis due to hemoglobin with high oxygen affinity	*HBA1* *HBA2* *HBB*	AD

***Key:** AD= Autosomal dominant; AR= Autosomal Recessive

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* gene, which is important in the production of new erythrocytes. Secondary congenital erythrocytosis can be due to pathogenic variants in genes of the hypoxia-sensing pathway which regulate erythropoietin production (*EGLN1, EPAS1, 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 Erythrocytosis Gene Sequencing panel uses a combination of Next-Generation Sequencing (NGS) and Sanger sequencing to detect pathogenic variants in nine genes that are known to cause erythrocytosis. We use NGS to detect variants in the *BPGM, EGLN1, EPAS1, EPOR, JAK2,* and *VHL* genes. We use Sanger sequencing to detect variants in the *HBA1, HBA2,* and *HBB* genes. We also offer single gene analysis and targeted variant analysis for any gene on the Erythrocytosis Gene Sequencing Panel.

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

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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:

Next Generation Sequencing (NGS) Panels: Testing for the *BPGM, EGLN1, EPAS1, EPOR, JAK2,* and *VHL* genes is performed by enrichment of the coding exons, flanking intronic and untranslated regions (5′ and 3′), as well as known pathogenic variants (HGMD 2018.1) in the promoter and deep intronic regions of the genes specified using oligonucleotide probe hybridization followed by next-generation sequencing with >50X 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. Regions with <50X will be filled in by Sanger sequencing. A detailed non-coding variant list is available upon request.

Gene Specific Sequencing: PCR-based sequencing of entire coding region, intron/exon boundaries, as well as known pathogenic variants (HGMD 2018.1) in the promoter and deep intronic regions of the specified gene. The *HBA1, HBA2,* and *HBB* genes are always performed by Gene Specific Sequencing due to high homologous regions.

Variant Specific Analysis: Sanger sequencing following PCR amplification of the targeted variant(s) of the specified gene.

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.

Limitations: Variants in regulatory regions and non-reported variants in untranslated regions may not be detected by this test. Large deletions/duplications involving an entire single exon or multiple exons and other complex genetic rearrangement events will not be identified using NGS methodology. Rare primer site variants may lead to erroneous results.

Note: Single gene sequencing is available for all genes on the panel.

Turn-Around Time:

- **Erythrocytosis Gene Sequencing Panel:** 28–42 days
- **Single Gene Sequencing:** 28 days

CPT Codes:

- **Erythrocytosis Gene Sequencing Panel:** 81404 x1, 81479 x3
- **Single Gene Testing and Targeted Variant 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 Friday.

Ship to:

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

References:

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Hong, Wan-Jen, and Jason Gotlib. "Hereditary erythrocytosis, thrombocytosis and neutrophilia." Best Practice & Research Clinical Haematology 27.2 (2014): 95-106.

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