Red Blood Cell Membrane Disorder Panel

Test code: HE1201

Is a 7 gene panel that includes assessment of non-coding variants.

Is ideal for patients with a clinical suspicion of elliptocytosis, hereditary spherocytosis or stomatocytosis. The genes on this panel are included in the Anemia Panel and the Comprehensive Hematology Panel.

Hereditary spherocytosis is clinically the most important disease in this group. It has an autosomal dominant pattern of inheritance in 75% of cases and the remaining are due to recessive and de novo mutations. This panel is included in the Anemia Panel and Comprehensive Hematology Panel.

About Red Blood Cell Membrane Disorder

The inherited disorders of the erythrocyte membrane include hereditary spherocytosis, hereditary elliptocytosis, hereditary pyropoikilocytosis and hereditary stomatocytosis. These all belong to a group of inherited hemolytic anemias. The clinical characteristics and underlying genetic mutations are very heterogenic from mutations leading to decreased red cell deformability, reduced life span and premature removal of the erythrocytes from the circulation. Hereditary spherocytosis is the most common congenital hemolytic anemia among Caucasians with an estimated prevalence ranging from 1:2,000 to 1:5,000. The most common genetic defect is due to mutations in ANK1 and the second most commonly in SPTB. The clinical manifestations can vary from mild to severe hemolytic anemia requiring exchange transfusions, variable jaundice, splenomegaly and cholelithiasis. Hereditary elliptocytosis is usually asymptomatic but approximately 10% of the cases may have moderate to severe anemia including the cases with the severe variant of pyropoikilocytosis. It is more common in patients originated from West Africa with the prevalence of 2%. Hereditary stomatocytosis is an autosomal dominant disorder characterized by the the dominance of stomatocytes in blood smear associated with moderate to severe anemia. Stomatocytes are sequestered by the spleen. Splenectomy is very beneficial in the treatment of hereditary spherocytosis and elliptocytosis but contraindicated in hereditary stomatocytosis, because it increases the risk of thromboembolic complications.

Availability

Results in 3-4 weeks

Gene set description

Genes in the Red Blood Cell Membrane Disorder Panel and their clinical significance

<table>
<thead>
<tr>
<th>Gene</th>
<th>Associated phenotypes</th>
<th>Inheritance</th>
<th>ClinVar</th>
<th>HGMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANK1</td>
<td>Spherocytosis</td>
<td>AD/AR</td>
<td>20</td>
<td>105</td>
</tr>
<tr>
<td>EPB41</td>
<td>Ellipsocytosis 1</td>
<td>AR</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>EPB42</td>
<td>Spherocytosis</td>
<td>AR</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>RHAG</td>
<td>Overhydrated hereditary stomatocytosis, Anemia, hemolytic, Rh-null, regulator type, Anemia, hemolytic,Rh-Mod type, RHAG blood group</td>
<td>AD/AR/BG</td>
<td>13</td>
<td>28</td>
</tr>
<tr>
<td>SLC4A1</td>
<td>Spherocytosis, Ovalcytosis, Renal tubular acidosis, distal, with hemolytic anemia, Cryohydrocytosis, Acanthocytosis, Band 3 Memphis</td>
<td>AD/AR/BG</td>
<td>38</td>
<td>122</td>
</tr>
<tr>
<td>SPTA1</td>
<td>Spherocytosis, Ellipsocytosis, Pyropoikilocytosis</td>
<td>AD/AR</td>
<td>29</td>
<td>51</td>
</tr>
<tr>
<td>SPTB</td>
<td>Spherocytosis, Anemia, neonatal hemolytic, Ellipsocytosis</td>
<td>AD/AR</td>
<td>24</td>
<td>99</td>
</tr>
</tbody>
</table>
*Some regions of the gene are duplicated in the genome. Read more.*

# The gene has suboptimal coverage (means <90% of the gene’s target nucleotides are covered at >20x with mapping quality score (MQ>20) reads), and/or the gene has exons listed under Test limitations section that are not included in the panel as they are not sufficiently covered with high quality sequence reads.

The sensitivity to detect variants may be limited in genes marked with an asterisk (*) or number sign (#)

Gene refers to the HGNC approved gene symbol; Inheritance refers to inheritance patterns such as autosomal dominant (AD), autosomal recessive (AR), mitochondrial (mi), X-linked (XL), X-linked dominant (XLD) and X-linked recessive (XLR); ClinVar refers to the number of variants in the gene classified as pathogenic or likely pathogenic in this database (ClinVar); HGMD refers to the number of variants with possible disease association in the gene listed in Human Gene Mutation Database (HGMD). The list of associated, gene specific phenotypes are generated from CGD or Mitomap databases.

### Non-coding disease causing variants covered by the panel

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genomic location HG19</th>
<th>HGVS</th>
<th>RefSeq</th>
<th>RS-number</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANK1</td>
<td>Chr8:41566510</td>
<td>c.1900-17G&gt;A</td>
<td>NM_001142446.1</td>
<td>rs786205243</td>
</tr>
<tr>
<td>ANK1</td>
<td>Chr8:41566511</td>
<td>c.1900-18C&gt;A</td>
<td>NM_001142446.1</td>
<td></td>
</tr>
<tr>
<td>ANK1</td>
<td>Chr8:41655127</td>
<td>c.-73_-72delTG</td>
<td>NM_020476.2</td>
<td>rs786205242</td>
</tr>
<tr>
<td>SLC4A1</td>
<td>Chr17:42340296</td>
<td>c.-62G&gt;A</td>
<td>NM_000342.3</td>
<td>rs387906565</td>
</tr>
<tr>
<td>SPTA1</td>
<td>Chr1:158613314</td>
<td>c.4339-99C&gt;T</td>
<td>NM_003126.2</td>
<td>rs200830867</td>
</tr>
<tr>
<td>SPTA1</td>
<td>Chr1:158626459</td>
<td>c.2806-13T&gt;G</td>
<td>NM_003126.2</td>
<td></td>
</tr>
</tbody>
</table>

### Test Strengths

The strengths of this test include:

- CAP accredited laboratory
- CLIA-certified personnel performing clinical testing in a CLIA-certified laboratory
- Powerful sequencing technologies, advanced target enrichment methods and precision bioinformatics pipelines ensure superior analytical performance
- Careful construction of clinically effective and scientifically justified gene panels
- Some of the panels include the whole mitochondrial genome (please see the Panel Content section)
- Our Nucleus online portal providing transparent and easy access to quality and performance data at the patient level
- Our publicly available analytic validation demonstrating complete details of test performance
- ~2,000 non-coding disease causing variants in our clinical grade NGS assay for panels (please see 'Non-coding disease causing variants covered by this panel' in the Panel Content section)
- Our rigorous variant classification scheme
- Our systematic clinical interpretation workflow using proprietary software enabling accurate and traceable processing of NGS data
- Our comprehensive clinical statements

### Test Limitations

This test does not detect the following:

- Complex inversions
- Gene conversions
- Balanced translocations
Some of the panels include the whole mitochondrial genome but not all (please see the Panel Content section).
Repeat expansion disorders unless specifically mentioned.
Non-coding variants deeper than ±20 base pairs from exon-intron boundary unless otherwise indicated (please see above Panel Content / non-coding variants covered by the panel).

**This test may not reliably detect the following:**
- Low level mosaicism in nuclear genes (variant with a minor allele fraction of 14.6% is detected with 90% probability).
- Stretches of mononucleotide repeats.
- Low level heteroplasmy in mtDNA (>90% are detected at 5% level).
- Indels larger than 50bp.
- Single exon deletions or duplications.
- Variants within pseudogene regions/duplicated segments.
- Some disease causing variants present in mtDNA are not detectable from blood, thus post-mitotic tissue such as skeletal muscle may be required for establishing molecular diagnosis.

The sensitivity of this test may be reduced if DNA is extracted by a laboratory other than Blueprint Genetics.

For additional information, please refer to the Test performance section and see our Analytic Validation.

**Test performance**

The Blueprint Genetics red blood cell membrane disorder panel covers classical genes associated with hereditary spherocytosis, elliptocytosis and stomatocytosis. The genes on the panel have been carefully selected based on scientific literature, mutation databases and our experience.

Our panels are sliced from our high-quality whole exome sequencing data. Please see our sequencing and detection performance table for different types of alterations at the whole exome level (Table).

Assays have been validated for different starting materials including EDTA-blood, isolated DNA (no FFPE), saliva and dry blood spots (filter card) and all provide high-quality results. The diagnostic yield varies substantially depending on the assay used, referring healthcare professional, hospital and country. Blueprint Genetics' Plus Analysis (Seq+Del/Dup) maximizes the chance to find a molecular genetic diagnosis for your patient although Sequence Analysis or Del/Dup Analysis may be a cost-effective first line test if your patient's phenotype is suggestive of a specific mutation type.

The genes on the panel have been carefully selected based on scientific literature, mutation databases and our experience.

Our panels are sectioned from our high-quality, clinical grade NGS assay. Please see our sequencing and detection performance table for details regarding our ability to detect different types of alterations (Table).

<table>
<thead>
<tr>
<th>Alteration Type</th>
<th>Sensitivity % (TP/(TP+FN))</th>
<th>Specificity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single nucleotide variants</td>
<td>99.89% (99,153/99,266)</td>
<td>&gt;99.9999%</td>
</tr>
<tr>
<td>Insertions, deletions and indels by sequence analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-10 bps</td>
<td>96.9% (7,563/7,806)</td>
<td>&gt;99.9999%</td>
</tr>
<tr>
<td>11-50 bps</td>
<td>99.13% (2,524/2,546)</td>
<td>&gt;99.9999%</td>
</tr>
<tr>
<td>Copy number variants (exon level dels/dups)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 exon level deletion (heterozygous)</td>
<td>100% (20/20)</td>
<td>NA</td>
</tr>
<tr>
<td>1 exon level deletion (homozygous)</td>
<td>100% (5/5)</td>
<td>NA</td>
</tr>
<tr>
<td>1 exon level deletion (het or homo)</td>
<td>100% (25/25)</td>
<td>NA</td>
</tr>
<tr>
<td>Simulation Type</td>
<td>Sensitivity %</td>
<td>Specificity %</td>
</tr>
<tr>
<td>-----------------------------------------------------</td>
<td>---------------</td>
<td>---------------</td>
</tr>
<tr>
<td>2-7 exon level deletion (het or homo)</td>
<td>100% (44/44)</td>
<td>NA</td>
</tr>
<tr>
<td>1-9 exon level duplication (het or homo)</td>
<td>75% (6/8)</td>
<td>NA</td>
</tr>
<tr>
<td>Simulated CNV detection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 exons level deletion/duplication</td>
<td>98.7%</td>
<td>100.00%</td>
</tr>
<tr>
<td>Microdeletion/-duplication sdrs (large CNVs, n=37))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size range (0.1-47 Mb)</td>
<td>100% (37/37)</td>
<td></td>
</tr>
</tbody>
</table>

The performance presented above reached by Blueprint Genetics high-quality, clinical grade NGS sequencing assay with the following coverage metrics:

- Mean sequencing depth: 143X
- Nucleotides with >20x sequencing coverage (%): 99.86%

Performance of Blueprint Genetics Mitochondrial Sequencing Assay:

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity %</th>
<th>Specificity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANALYTIC VALIDATION (NA samples; n=4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single nucleotide variants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heteroplasmic (45-100%)</td>
<td>100.0% (50/50)</td>
<td>100.0%</td>
</tr>
<tr>
<td>Heteroplasmic (35-45%)</td>
<td>100.0% (87/87)</td>
<td>100.0%</td>
</tr>
<tr>
<td>Heteroplasmic (25-35%)</td>
<td>100.0% (73/73)</td>
<td>100.0%</td>
</tr>
<tr>
<td>Heteroplasmic (15-25%)</td>
<td>100.0% (77/77)</td>
<td>100.0%</td>
</tr>
<tr>
<td>Heteroplasmic (10-15%)</td>
<td>100.0% (74/74)</td>
<td>100.0%</td>
</tr>
<tr>
<td>Heteroplasmic (5-10%)</td>
<td>100.0% (3/3)</td>
<td>100.0%</td>
</tr>
<tr>
<td>Heteroplasmic (&lt;5%)</td>
<td>50.0% (2/4)</td>
<td>100.0%</td>
</tr>
<tr>
<td>CLINICAL VALIDATION (n=76 samples)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All types</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single nucleotide variants n=2026 SNVs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heteroplasmic (45-100%)</td>
<td>100.0% (1940/1940)</td>
<td>100.0%</td>
</tr>
<tr>
<td>Heteroplasmic (35-45%)</td>
<td>100.0% (4/4)</td>
<td>100.0%</td>
</tr>
<tr>
<td>Heteroplasmic (25-35%)</td>
<td>100.0% (3/3)</td>
<td>100.0%</td>
</tr>
<tr>
<td>Heteroplasmic (15-25%)</td>
<td>100.0% (3/3)</td>
<td>100.0%</td>
</tr>
<tr>
<td>Heteroplasmic (10-15%)</td>
<td>100.0% (9/9)</td>
<td>100.0%</td>
</tr>
</tbody>
</table>
### Heteroplasmic (5-10%)
- 92.3% (12/13)
- 99.98%

### Heteroplasmic (<5%)
- 88.9% (48/54)
- 99.93%

### Insertions and deletions by sequence analysis n=40 indels
- Heteroplasmic (45-100%) 1-10bp
  - 100.0% (32/32)
  - 100.0%
- Heteroplasmic (5-45%) 1-10bp
  - 100.0% (3/3)
  - 100.0%
- Heteroplasmic (<5%) 1-10bp
  - 100.0% (5/5)
  - 99.997%

### SIMULATION DATA /mitomap mutations
- Insertions, and deletions 1-24 bps by sequence analysis; n=17
  - Homoplasmic (100%) 1-24bp
    - 100.0% (17/17)
    - 99.98%
  - Heteroplasmic (50%)
    - 100.0% (17/17)
    - 99.99%
  - Heteroplasmic (25%)
    - 100.0% (17/17)
    - 100.0%
  - Heteroplasmic (20%)
    - 100.0% (17/17)
    - 100.0%
  - Heteroplasmic (15%)
    - 100.0% (17/17)
    - 100.0%
  - Heteroplasmic (10%)
    - 94.1% (16/17)
    - 100.0%
  - Heteroplasmic (5%)
    - 94.1% (16/17)
    - 100.0%

### Copy number variants (separate artificial mutations; n=1500)
- Homoplasmic (100%) 500 bp, 1kb, 5 kb
  - 100.0%
  - 100.0%
- Heteroplasmic (50%) 500 bp, 1kb, 5 kb
  - 100.0%
  - 100.0%
- Heteroplasmic (30%) 500 bp, 1kb, 5 kb
  - 100.0%
  - 100.0%
- Heteroplasmic (20%) 500 bp, 1kb, 5 kb
  - 99.7%
  - 100.0%
- Heteroplasmic (10%) 500 bp, 1kb, 5 kb
  - 99.0%
  - 100.0%

### The performance presented above reached by following coverage metrics at assay level (n=66)

<table>
<thead>
<tr>
<th>Metric</th>
<th>Mean of medians</th>
<th>Median of medians</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean sequencing depth MQ0 (clinical)</td>
<td>18224X</td>
<td>17366X</td>
</tr>
<tr>
<td>Nucleotides with &gt;1000x MQ0 sequencing coverage (%) (clinical)</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>rho zero cell line (=no mtDNA), mean sequencing depth</td>
<td>12X</td>
<td></td>
</tr>
</tbody>
</table>

### Bioinformatics

The target region for each gene includes coding exons and ±20 base pairs from the exon-intron boundary. In addition, the panel includes non-coding variants if listed above (Non-coding variants covered by the panel). Some regions of the gene(s) may be removed from the panel if specifically mentioned in the "Test limitations" section above. The sequencing data generated in our laboratory is analyzed with our proprietary data analysis and annotation pipeline, integrating state-of-the-art data analysis tools.
Blueprint Genetics

algorithms and industry-standard software solutions. Incorporation of rigorous quality control steps throughout the workflow of the pipeline ensures the consistency, validity and accuracy of results. Our pipeline is streamlined to maximize sensitivity without sacrificing specificity. We have incorporated a number of reference population databases and mutation databases such as, but not limited to, 1000 Genomes Project, gnomAD, ClinVar and HGMD into our clinical interpretation software to make the process effective and efficient. For missense variants, in silico variant prediction tools such as SIFT, PolyPhen, MutationTaster are used to assist with variant classification. Through our online ordering and statement reporting system, Nucleus, the customer has an access to details of the analysis, including patient specific sequencing metrics, a gene level coverage plot and a list of regions with inadequate coverage if present. This reflects our mission to build fully transparent diagnostics where customers have easy access to crucial details of the analysis process.

Clinical interpretation

We provide customers with the most comprehensive clinical report available on the market. Clinical interpretation requires a fundamental understanding of clinical genetics and genetic principles. At Blueprint Genetics, our PhD molecular geneticists, medical geneticists and clinical consultants prepare the clinical statement together by evaluating the identified variants in the context of the phenotypic information provided in the requisition form. Our goal is to provide clinically meaningful statements that are understandable for all medical professionals regardless of whether they have formal training in genetics.

Variant classification is the corner stone of clinical interpretation and resulting patient management decisions. Our classifications follow the Blueprint Genetics Variant Classification Schemes based on the ACMG guideline 2015. Minor modifications were made to increase reproducibility of the variant classification and improve the clinical validity of the report. Our experience with tens of thousands of clinical cases analyzed at our laboratory allowed us to further develop the industry standard.

The final step in the analysis of sequence variants is confirmation of variants classified as pathogenic or likely pathogenic using bi-directional Sanger sequencing. Variant(s) fulfilling the following criteria are not Sanger confirmed: the variant quality score is above the internal threshold for a true positive call, and visual check-up of the variant at IGV is in-line with the variant call. Reported variants of uncertain significance are confirmed with bi-directional Sanger sequencing only if the quality score is below our internally defined quality score for true positive call. Reported copy number variations with a size <10 exons are confirmed by orthogonal methods such as qPCR if the specific CNV has been seen less than three times at Blueprint Genetics.

Our clinical statement includes tables for sequencing and copy number variants that include basic variant information (genomic coordinates, HGVS nomenclature, zygosity, allele frequencies, in silico predictions, OMIM phenotypes and classification of the variant). In addition, the statement includes detailed descriptions of the variant, gene and phenotype(s) including the role of the specific gene in human disease, the mutation profile, information about the gene’s variation in population cohorts and detailed information about related phenotypes. We also provide links to the references used, congress abstracts and mutation variant databases used to help our customers further evaluate the reported findings if desired. The conclusion summarizes all of the existing information and provides our rationale for the classification of the variant.

Identification of pathogenic or likely pathogenic variants in dominant disorders or their combinations in different alleles in recessive disorders are considered molecular confirmation of the clinical diagnosis. In these cases, family member testing can be used for risk stratification within the family. In the case of variants of uncertain significance (VUS), we do not recommend family member risk stratification based on the VUS result. Furthermore, in the case of VUS, we do not recommend the use of genetic information in patient management or genetic counseling.

Our interpretation team analyzes millions of variants from thousands of individuals with rare diseases. Thus, our database, and our understanding of variants and related phenotypes, is growing by leaps and bounds. Our laboratory is therefore well positioned to re-classify previously reported variants as new information becomes available. If a variant previously reported by Blueprint Genetics is re-classified, our laboratory will issue a follow-up statement to the original ordering health care provider at no additional cost.

#}

ICD codes

Commonly used ICD-10 codes when ordering the Red Blood Cell Membrane Disorder Panel
ICD-10          Disease

D58.0          Hereditary spherocytosis
D58.1          Elliptocytosis
D58.8          Stomatocytosis

Accepted sample types

- EDTA blood, min. 1 ml
- Purified DNA, min. 3μg*
- Saliva (Oragene DNA OG-500 kit)

Label the sample tube with your patient’s name, date of birth and the date of sample collection.

Note that we do not accept DNA samples isolated from formalin-fixed paraffin-embedded (FFPE) tissue.

Resources

- GeneReviews - *EPB42*-Related Hereditary Spherocytosis
- GeneReviews - EPB42-Related Hereditary Spherocytosis
- NORD - Hereditary Spherocytosis
- Orphanet - Hereditary Elliptocytosis
- Orphanet - Hereditary Stomatocytosis
- Patient Information UK - Hereditary Spherocytosis