Blueprint Genetics

Hyperphenylalaninemia Panel

Test code: ME2001

Is ideal for patients with a clinical suspicion of hyperphenylalaninaemias including hyperphenylalaninemia due to BH4 deficiency. The genes on this panel are included in the Comprehansive Metabolism Panel.

Hyperphenylalaninemias (HPA) including phenylketonuria (PKU) and tetrahydrobiopterin (BH4) deficiency are inherited in autosomal recessive manner. Clinical utility of this Panel for PKU is estimated at 95-100%. This Panel is included in the Comprehensive Metabolism Panel.

About Hyperphenylalaninemia

Hyperphenylalaninemias (HPA) are errors in metabolism resulting in characterics of elevated levels of phenylalanine amino acid in the blood. Phenylketonuria (PKU) results in hyperphenylalaninemia if left untreated. Elevated levels if phenylalanine will make a severe risk of intellectual disability for a child. Unborn babies with mutation in homozygous state are unaffected as mother's circulation prevents buildup. After birth, phenylalanine-restricted diet prevents intellectual problems and the persons with homozygous mutated genotype have normal mental development. However, maternal PKU without metabolic control predisposes babies to severe mental retardation and heart defects. This is an example of a genetic disease of a baby based on mother's genotype. Classical PKU is caused by mutations in PAH, but some 2% of all HPAs result from impaired synthesis or recycling of tetrahydrobiopterin (BH4). Causative mutations in these cases are in *GCH1*, *PCBD1*, *PTS* or *QDPR* genes. The worldwide prevalence of PKU is estimated at 1:10 000 births having, however, rather big variation in different populations. The prevalence of tetrahydrobiopterin is estimated at <1:500 000 newborns. However, in certain populations (Saudi Arabia, Taiwan, Turkey, China) HPA is more commonly associated with shortage of tetrahydrobiopterin than with classical PKU.

Availability

Results in 3-4 weeks

Gene set description

Genes in the Hyperphenylalaninemia Panel and their clinical significance

Gene	Associated phenotypes	Inheritance	ClinVar	HGMD
DNAJC12	Hyperphenylalaninemia, mild, non-BH4-deficient, Dystonia, Other hyperphenylalaninemias	AR	3	8
GCH1	Dopa-Responsive Dystonia Hyperphenylalaninemia, BH4-deficient, GTP Cyclohydrolase 1-Deficient Dopa-Responsive Dystonia	AD/AR	48	240
PAH	Hyperphenylalaninemia, non-PKU mild, Phenylketonuria	AR	294	966
PCBD1	Hyperphenylalaninemia, BH4-deficient	AR	6	11
PTS	Hyperphenylalaninemia, BH4-deficient	AR	34	112
QDPR	Hyperphenylalaninemia, BH4-deficient	AR	14	66

*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

Gene	Genomic location HG19	HGVS	RefSeq	RS-number
GCH1	Chr14:55369403	c.-22C>T	NM_000161.2	
PAH	Chr12:103232809	c.*144A>G	NM_000277.1	rs375319584
PAH	Chr12:103237404	c.1199+20G>C	NM_000277.1	rs62509018
PAH	Chr12:103237407	c.1199+17G>A	NM_000277.1	rs62508613
PAH	Chr12:103237568	c.1066-11G>A	NM_000277.1	rs5030855
PAH	Chr12:103237568	c.1066-12delT	NM_000277.1	
PAH	Chr12:103237570	c.1066-13T>G	NM_000277.1	
PAH	Chr12:103237571	c.1066-14C>G	NM_000277.1	rs62507334
PAH	Chr12:103238075	c.1065+39G>T	NM_000277.1	rs62510582
PAH	Chr12:103260355	c.509+15_509+18delCTTG	NM_000277.1	rs1335303703
PAH	Chr12:103288709	c.169-13T>G	NM_000277.1	rs62507341
PTS	Chr11:112098994	c.84-323A>T	NM_000317.2	rs794726657
PTS	Chr11:112099026	c.84-291A>G	NM_000317.2	
PTS	Chr11:112100215	c.164-716A>T	NM_000317.2	
PTS	Chr11:112101310	c.187-38dupG	NM_000317.2	
QDPR	Chr4:17500790	c.436+2552A>G	NM_000320.2	

Test Strengths

The clinical utility of this panel for hyperphenylalanemia is estimated at 95-100%.

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 hyperphenylalaninemia panel covers classical genes associated with classical phenylketonuria, other hyperphenylalaninemias and hyperphenylalaninemia due to BH4 deficiency. 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).

	Sensitivity % (TP/(TP+FN)	Specificity %

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

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.

	Sensitivity % (TP/(TP+FN)	Specificity
ANALYTIC VALIDATION (NA samples; n=4)		
Single nucleotide variants		
Heteroplasmic (45-100%)	100.0% (50/50)	100.0%
Heteroplasmic (35-45%)	100.0% (87/87)	100.0%
Heteroplasmic (25-35%)	100.0% (73/73)	100.0%
Heteroplasmic (15-25%)	100.0% (77/77)	100.0%
Heteroplasmic (10-15%)	100.0% (74/74)	100.0%
Heteroplasmic (5-10%)	100.0% (3/3)	100.0%
Heteroplasmic (<5%)	50.0% (2/4)	100.0%

CLINICAL VALIDATION (n=76 samples)		
All types		
Single nucleotide variants n=2084 SNVs		
Heteroplasmic (45-100%)	100.0% (1940/1940)	100.0%
Heteroplasmic (35-45%)	100.0% (4/4)	100.0%
Heteroplasmic (25-35%)	100.0% (3/3)	100.0%
Heteroplasmic (15-25%)	100.0% (3/3)	100.0%
Heteroplasmic (10-15%)	100.0% (9/9)	100.0%
Heteroplasmic (5-10%)	92.3%(12/13)	99.98%
Heteroplasmic (<5%)	88.7% (47/53)	99.93%
Insertions and deletions by sequence analysis n=42 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)	>0.9999
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 artifical 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)		

	Mean of medians	Median of medians
Mean sequencing depth MQ0 (clinical)	18224X	17366X
Nucleotides with >1000x MQ0 sequencing coverage (%) (clinical)	100%	
rho zero cell line (=no mtDNA), mean sequencing depth	12X	

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 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.

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ICD codes

Commonly used ICD-10 codes when ordering the Hyperphenylalaninemia Panel

ICD-10	Disease
E70.0	Classical phenylketonuria
E70.1	Other hyperphenylalaninemias

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

- Children's PKU Network
- Cook for Love
- GeneReviews - Phenylalanine Hydroxylase Deficiency
- GeneReviews - Phenylketonuria
- NORD - Phenylketonuria
- NORD - Tetrahydrobiopterin Deficiency
- National PKU Alliance
- National PKU News
- PKU Foundation