

## Hereditary Hemorrhagic Telangiectasia (HHT) Panel

Test code: CA0201

Is ideal for patients with a clinical suspicion of hereditary hemorrhagic telangiectasia (HHT).

Hereditary hemorrhagic telangiectasia is inherited in an autosomal dominant pattern. Genetic diagnosis can confirm the clinical diagnosis and establish the subtype of hereditary hemorrhagic telangiectasia. Genetic diagnosis can then guide risk assessment and treatment strategies. Identifying the genetic cause enables risk assessment among family members. HHT Panel is included in the Pulmonary Arterial Hypertension Panel.

### About Hereditary Hemorrhagic Telangiectasia (HHT)

Hereditary hemorrhagic telangiectasia (HHT) is characterized by the presence of multiple arteriovenous malformations (AVMs) that lack intervening capillaries and result in direct connections between arteries and veins (GeneReviews). Small AVMs in vessels near the surface of the skin are visible and known as telangiectases. The extra pressure tends to enlarge these blood vessels, and may result in compression or irritation of adjacent tissues and frequent episodes of severe bleeding. Nosebleeds are very common in people with HHT. More serious problems may arise from hemorrhages in the brain, lungs, liver, or other organs. Hereditary hemorrhagic telangiectasia is widely distributed, occurring in many ethnic groups. It's prevalence is estimated to be 1 in 5,000-10,000.

### Availability

Results in 3-4 weeks

### Gene set description

Genes in the Hereditary Hemorrhagic Telangiectasia (HHT) Panel and their clinical significance

Gene	Associated phenotypes	Inheritance	ClinVar	HGMD
ACVRL1	Hereditary hemorrhagic telangiectasia	AD	140	430
ENG	Juvenile polyposis syndrome, Hereditary hemorrhagic telangiectasia	AD	158	491
EPHB4	Hydrops fetalis, nonimmune, and/or atrial septal defect, Capillary malformation-arteriovenous malformation	AD	1	51
GDF2	Hereditary hemorrhagic telangiectasia, type 5, Pulmonary arterial hypertension (PAH)	AD	3	17
<u>RASA1</u> #	Parkes Weber syndrome, Capillary malformation-arteriovenous malformation, Spinal arteriovenous anomalies	AD	55	132
SMAD4	Juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome, Polyposis, juvenile intestinal, Myhre dysplasia, Hereditary hemorrhagic telangiectasia	AD	179	143

\*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 (#)

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Gene refers to the HGNC approved gene symbol; Inheritance refers to inheritance patterns such as autosomal dominant (AD), autosomal recessive (AR), 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 [Orphanet](#) databases.

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

Gene	Genomic location HG19	HGVS	RefSeq	RS-number
ACVRL1	Chr12:52314269	c.1378-274C>G	NM_000020.2	
ACVRL1	Chr12:52314327	c.1378-216C>G	NM_000020.2	
ACVRL1	Chr12:52314387	c.1378-156_1378-155invCT	NM_000020.2	
ACVRL1	Chr12:52314412	c.1378-131C>G	NM_000020.2	
ACVRL1	Chr12:52314465	c.1378-78T>G	NM_000020.2	
ACVRL1	Chr12:52314474	c.1378-69C>A	NM_000020.2	
ENG	Chr9:130578354	c.1742-22T>C	NM_001114753.2	
ENG	Chr9:130588962	c.361-11T>A	NM_001114753.2	
ENG	Chr9:130616692	c.-58G>A	NM_001114753.2	rs971268057
ENG	Chr9:130616761	c.-127C>T	NM_001114753.2	
ENG	Chr9:130616776	c.-142A>T	NM_001114753.2	

## 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
- 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

Genes with suboptimal coverage in our assay are marked with number sign (#). Gene is considered to have suboptimal coverage when >90% of the gene's target nucleotides are not covered at >20x with mapping quality score (MQ>20) reads. The technology may have limited sensitivity to detect variants in genes marked with these symbols (please see the Panel content

table above).

**This test does not detect the following:**

- Complex inversions
- Gene conversions
- Balanced translocations
- Mitochondrial DNA variants
- 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 (variant with a minor allele fraction of 14.6% is detected with 90% probability)
- Stretches of mononucleotide repeats
- Indels larger than 50bp
- Single exon deletions or duplications
- Variants within pseudogene regions/duplicated segments

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

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).<sup>1</sup>

Assays have been validated for various sample types including EDTA-blood, isolated DNA (excluding from formalin fixed paraffin embedded tissue), saliva and dry blood spots (filter cards). These sample types were selected in order to maximize the likelihood for high-quality DNA yield. The diagnostic yield varies depending on the assay used, referring healthcare professional, hospital and country. Plus analysis increases the likelihood of finding a genetic diagnosis for your patient, as large deletions and duplications cannot be detected using sequence analysis alone. Blueprint Genetics’ Plus Analysis is a combination of both sequencing and deletion/duplication (copy number variant (CNV)) analysis.

## Performance of Blueprint Genetics high-quality, clinical grade NGS sequencing assay for panels.

	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	99.2% (7,745/7,806)	>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

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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%
Size range (0.1-47 Mb)	100% (25/25)	

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%

## Bioinformatics

The target region for each gene includes coding exons and  $\pm 20$  base pairs from the exon-intron boundary. In addition, the panel includes non-coding and regulatory 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 including, 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, ordering providers have access to the details of the analysis, including patient specific sequencing metrics, a gene level coverage plot and a list of regions with <20X sequencing depth if applicable. This reflects our mission to build fully transparent diagnostics where ordering providers can easily visualize the 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 is orthogonal confirmation. Sequence variants classified as pathogenic, likely pathogenic and variants of uncertain significance (VUS) are confirmed using bi-directional Sanger sequencing when they do not meet our stringent NGS quality metrics for a true positive call. □ Reported heterozygous and homo/hemizygous copy number variations with a size <10 and <3 target exons are confirmed by orthogonal methods such as qPCR if the specific CNV has been seen and

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confirmed 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, abstracts and variant databases used to help ordering providers 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. We do not recommend using variants of uncertain significance (VUS) for family member risk stratification or patient management. Genetic counseling is recommended.

Our interpretation team analyzes millions of variants from thousands of individuals with rare diseases. Our internal database and our understanding of variants and related phenotypes increases with every case analyzed. 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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## 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

- [Faughnan ME et al. International guidelines for the diagnosis and management of hereditary haemorrhagic telangiectasia. J Med Genet. 2011 Feb;48\(2\):73-87.](#)
- [GeneReviews](#)
- [GeneReviews-HHT](#)
- [HHT Foundation](#)
- [NORD](#)
- [NORD - HHT](#)