Hereditary Hemorrhagic Telangiectasia Panel

Versiti offers comprehensive genetic analysis to detect sequence variants and large deletions and duplications in six genes known to cause hereditary hemorrhagic telangiectasia. This panel can be ordered as:
- Next generation sequencing (NGS) only;
- NGS with reflex to array Comparative Genomic Hybridization (aCGH) deletion/duplication analysis if clinically significant variants explaining the patient's phenotype are not detected by sequencing;
- NGS with concurrent aCGH deletion/duplication analysis (both testing methodologies performed simultaneously); or
- Deletion/duplication analysis by aCGH only.

Hereditary hemorrhagic telangiectasia (HHT), previously known as Rendu-Osler-Weber disease, is a group of autosomal dominant disorders affecting vascular formation, due to defects in the transforming growth factor beta (TGF-β) or Ras-MAPK signaling pathways. Six genes have been identified as causative of or having significant phenotypic overlap with HHT. The established clinical diagnostic criteria (Curaçao criteria) include spontaneous and recurrent nosebleeds (epistaxis), multiple telangiectasias in characteristic locations (including face and fingers), visceral arteriovenous malformation (AVM), and family history of HHT in a first-degree relative. A clinical diagnosis of HHT is considered definite when three or more criteria are identified in a patient, and considered possible or suspected when two or more criteria are present. However, since symptoms of HHT often present in adolescence, the clinical diagnostic criteria are not sensitive in identifying affected children.

The worldwide prevalence of HHT is estimated at 1 in 5,000, though there is increased incidence in specific geographic locations including the Dutch Antilles islands; Funen Island, Denmark; the French region of Ain; Vermont, USA; Newcastle, UK; and Las Palmas de Gran Canaria, Grand Canary Island, Spain.

Given delayed presentation of clinical features in HHT and similar presentations of other genetic conditions, genetic testing identifies at-risk children who may not yet meet HHT diagnostic criteria. Precise diagnosis also guides personalized treatment plans, critical for a subset of patients with features of HHT at risk for additional clinical findings requiring unique surveillance or treatment. Gastrointestinal polyposis, pulmonary arterial hypertension, and Parkes Weber syndrome (leading to soft tissue and skeletal overgrowth) are seen in some HHT and HHT-like disorders.

The next generation sequencing (NGS) panel evaluates single nucleotide variants and small deletions and duplications, which are most commonly responsible for genetic disease. However, larger deletions and duplications, also referred to as copy number variations (CNVs), are a known cause of genetic disorders, but can escape detection by NGS analysis. Additional testing with aCGH deletion/duplication analysis is available for all genes on this panel to evaluate for CNVs encompassing one or more exons or affecting an entire gene.

Refer to the table for further information about each gene in the Hereditary Hemorrhagic Telangiectasia Panel, including the clinical phenotype and inheritance pattern.

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Hereditary Hemorrhagic Telangiectasia Panel: gene, clinical phenotype and inheritance pattern		
Gene	Clinical Phenotype	Inheritance
ACVRL1	**Hereditary hemorrhagic telangiectasia type 2:** epistaxis, mucocutaneous telangiectasias, and arteriovenous malformations (AVMs). Variants in *ACVRL1* are also reported in patients with pulmonary arterial hypertension.	Autosomal Dominant
ENG	**Hereditary hemorrhagic telangiectasia type 1:** epistaxis, mucocutaneous telangiectasias, and AVMs.	Autosomal Dominant
*EPHB4**	**Capillary malformation-arteriovenous malformation 2:** capillary malformations on the face or limbs, telangiectasias, epistaxis, and arteriovenous malformations or fistulas.	Autosomal Dominant
GDF2	**Hereditary hemorrhagic telangiectasia type 5:** epistaxis, mucocutaneous telangiectasias, and AVMs. *GDF2*-related vascular-anomaly syndrome: pulmonary arterial hypertension, cerebral AVMs, telangiectasias, and epistaxis.	Autosomal Dominant
*RASA1**	**Capillary malformation-arteriovenous malformation 1:** capillary malformations on the face or limbs, and arteriovenous malformations or fistulas.	Autosomal Dominant
SMAD4	**Juvenile polyposis syndrome (JPS):** predisposition to gastrointestinal hamartomatous polyps, and often seen along with features of HHT, characterized by epistaxis, mucocutaneous telangiectasias, and AVMs. Pathogenic gain-of-function variants in *SMAD4* cause Myhre syndrome, characterized by growth deficiency, intellectual disability, facial dysmorphisms, skeletal anomalies, and cardiovascular defects.	Autosomal Dominant

* Somatic pathogenic variants in *EPHB4* and *RASA1* have been described and associated with Parkes Weber syndrome. Please note that this assay is not designed for detection of somatic variants. See Assay Sensitivity and Limitations below.

Indications for testing:

Hereditary Hemorrhagic Telangiectasia Panel (NGS and/or aCGH), order code 4895

The Hereditary Hemorrhagic Telangiectasia Panel should be considered for:

- Confirmation of the diagnosis in individuals with clinical features suggestive of HHT to allow for accurate prognostication and appropriate surveillance plan to reduce morbidity and mortality
- Diagnosis of at-risk relatives with a family history of HHT with unknown genetic etiology

Single Gene Analysis (order code 4855) or Custom Blood Disorder Panel (order code 4850), (NGS and/or aCGH)

Analysis of genes included in this panel may also be ordered as a standalone Single Gene Analysis or as a Custom Blood Disorder Panel (2-10 genes), by NGS and/or by aCGH, as dictated by the patient's clinical and laboratory phenotype, as well as their ancestry, or to supplement previous genetic testing.

Targeted Familial Variant Analysis (order code 4970)

Targeted variant analysis for clinical diagnosis or prenatal diagnosis can also be performed on any gene in the panel when the pathogenic variant is known in the family. If the proband was not tested at Versiti, a control sample is preferred and may be required (please call the laboratory to discuss). If the familial variant is a large deletion or duplication (>20 base pairs), aCGH for the involved gene is required.

For clinical questions about laboratory tests and test utilization support, contact Versiti Client Services: (414) 937-6396 or 800-245-3117, option 1, to be directed to our genetic counselors and clinical support team.

Informed Consent:

It is recommended that healthcare providers obtain informed consent from the patient when genetic testing is ordered, consistent with any applicable state laws and regulations, documenting that the patient has been advised of and understands the indications for and implications of the genetic test. This panel is designed for clinical detection of germline genetic variants in genes with strong or definitive evidence for causality of hereditary hemorrhagic telangiectasia. Test results may nonetheless yield genetic findings that may be unrelated to the current clinical presentation, and/or may carry individual or familial implications such as risk for syndromic manifestation, predisposition to malignancy, and/or reproductive implications (such as carrier status). If needed, an informed consent form for Versiti Hematology Genetics testing can be found at http://www.versiti.org/hg under forms.

Test method:

NGS: This next generation sequencing assay analyzes the complete coding region of six genes plus a minimum 30bp of non-coding DNA, including intron-exon boundaries, and is compared to the build GRCh37.p13 reference sequence. Analysis of *ACVRL1* and *ENG* also includes the 3' UTR. These targeted regions are captured by hybridization, amplified, and sequenced by massively parallel sequencing. Regions will have a minimum coverage of 50x and those regions with less than 50 sequencing reads or low-quality coverage are supplemented with Sanger sequencing. All regions are covered by bidirectional analysis. Variants are identified by a customized bioinformatics pipeline, analyzed and comprehensively interpreted by our team of practicing hematologists with expertise in non-malignant hematology and laboratory diagnostics, scientists, and genetic counselors. All reported variants, including pathogenic, likely pathogenic, and variants of uncertain significance, are confirmed by Sanger sequencing. For prenatal testing, analysis of variable number tandem repeats (VNTR) is used to confirm results are not affected by maternal cell contamination.

aCGH: The specific genes are analyzed for copy number variations due to deletion or duplication by high density gene-focused array Comparative Genomic Hybridization. Probes are approximately 60bp in length and density of coverage in exonic regions is a minimum of 4 probes per 500 bp. Genomic DNA for the samples and sex-matched references are denatured, labeled with fluorescent dye and hybridized, the array is washed and scanned, and analysis is performed for the specific genes requested.

Assay sensitivity and limitations:

NGS: The analytical sensitivity of the NGS test is >99% for single nucleotide changes and insertions and deletions of less than 20 bp. NGS analysis is not designed to detect large deletions or duplications (>20 bp), or variants that are outside of the regions sequenced. Low level mosaicism will not be detected by this sequencing methodology.

aCGH: Balanced chromosomal rearrangements (i.e., translocations, inversions) or point mutations that may be the cause of the clinical phenotype cannot be detected vis aCGH. Any exonic deletion or duplication smaller than 500bp may not be detected. Low level mosaicism will not be detected by aCGH. Probe performance could be affected by multiple SNPs in a given region. Breakpoints occurring outside the targeted gene(s) will not be defined.

Clinical sensitivity:

The clinical sensitivity of comprehensive genetic testing (NGS and aCGH) of the six genes known to be associated with hereditary hemorrhagic telangiectasia is highest in patients presenting with recurrent epistaxis, telangiectasias and arteriovenous malformations affecting multiple organs and in the context of a family history of a similar clinical phenotype.

Reporting of results:

Results are classified and reported in accordance with ACMG next generation sequencing and copy number variation standards. Sequence variants and large deletions and duplications predicted to be pathogenic, likely pathogenic, and of uncertain significance will be reported; variants classified as likely benign or benign are typically not reported but such data are available upon request. Sequence variants are described using standard Human Genome Variation Society (HGVS) nomenclature (http://hgvs.org); copy number variants are described in accordance with the International System for Human Cytogenomic Nomenclature (ISCN).

Specimen requirements:

Parental/Patient/Pediatric: 3-5 mL Whole blood (EDTA tube, lavender top), 2-5 mL Bone marrow (EDTA tube, lavender top), 3-4 Buccal swabs, or ≥1 ug of DNA at ≥50 ng/uL of high-quality DNA.

Fetal: 7-15 mL amniotic fluid, 5-10 mg chorionic villi; back-up culture of amniocytes or chorionic villi is highly recommended. Cultured: Two T25 flasks cultured amniocytes or chorionic villi ($2x10^6$ minimum). Maternal blood sample of 3-5 mL Whole blood (EDTA tube, lavender top) is requested for all prenatal samples for maternal cell contamination studies. For questions, please contact the laboratory to discuss sample requirements.

SHIP

Shipping requirements:

Ship on an ice pack or at room temperature. Protect from freezing. Place the specimen and the requisition into plastic bags and seal. Insert into a Styrofoam container, seal and place into a sturdy cardboard box, and tape securely. Ship the package in compliance with your overnight carrier guidelines. Label with the following address:

Client Services/Diagnostic Laboratory
Versiti
638 N. 18th St
Milwaukee, WI, 53233

ORDER

Required forms:

Please complete all pages of the requisition form. Clinical history (including patient-reported ancestry, clinical diagnosis, family history, and relevant laboratory findings) is necessary for optimal interpretation of genetic test results and recommendations.

Clinical and laboratory history can either be recorded on the requisition form or clinical and laboratory reports can be submitted with the sample.

CPT Codes/Billing/Turnaround time:

Test code: 4895

CPT codes: For recommended CPT codes, visit the Versiti.org/test menu

Turnaround time: NGS only, aCGH only, or NGS and aCGH concurrently: 21 days

NGS reflex to aCGH: 21 days (if NGS only, aCGH not needed) or 42 days (with reflex to aCGH)

The CPT codes provided are subject to change as more information becomes available. CPT codes are provided only as guidance to assist clients with billing.

For additional information related to shipping, billing or pricing, please contact, Versiti Client Services: (414) 937-6396 or 800-245-3117, Option 1, or labinfo@versiti.org.

References:

Hereditary hemorrhagic telangiectasia references

1. Bayrak-Toydemir P, Stevenson DA. Capillary Malformation-Arteriovenous Malformation Syndrome. 2011 Feb 22 [updated 2019 Sep 12]. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993–2023.
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Variant interpretation references

1. Bean LJH, Funke B, Carlston CM, et al. Diagnostic gene sequencing panels: from design to report-a technical standard of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2020;22(3):453-461. doi:10.1038/s41436-019-0666-z
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3. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-424. doi:10.1038/gim.2015.30
4. Riggs ER, Andersen EF, Cherry AM, et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). Genet Med. 2020;22(2):245-257. doi:10.1038/s41436-019-0686-8.

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