UAB MEDICAL GENOMICS LABORATORY

RNA-based *NF1* and gDNA-based *SPRED1* Testing on Blood (NFSP-R)

Ordering Information

Acceptable specimen types:

- Fresh blood sample (3-6ml EDTA; to arrive <60-70 hours after collection)

  Saliva or DNA are NOT acceptable specimens

Turnaround time:

22 working days

Price, CPT codes, and Z code:

$2,000 (USD – institutional/self-pay);

CPT: 88230, 81408, 81479 (x2), and 81405

Z code: ZB6AJ

Candidates for this test:

Patients who need the most sensitive and specific test with the fastest turnaround time

Specimen shipping and handling:

- Please find acceptable specimen type above.
- Blood specimens must be kept at room temperature and received within 60-72 hours of collection.
- Submitted samples must arrive within the laboratory between Monday-Friday.
- All submitted specimens must be sent at room temperature. DO NOT ship on ice.

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Birmingham, Alabama 35294-0005 Fax: (205) 996-2929
www.uab.edu/medicine/genetics/medical-genomics-laboratory

- Specimens must be packaged to prevent breakage and absorbent material must be included in the package to absorb liquids in the event that breakage occurs. Also, the package must be shipped in double watertight containers (e.g. a specimen pouch + the shipping company's diagnostic envelope).
- To request a sample collection kit, please visit the website or email medgenomics@uabmc.edu to complete the specimen request form.
- Please contact the MGL (via email at medgenomics@uabmc.edu, or via phone at 205-934-5562) prior to sample shipment and provide us with the date of shipment and tracking number of the package so that we can better ensure receipt of the samples.

Required forms:

- Test Requisition Form
- Form for Customs (for international shipments)

Note: Detailed and accurate completion of this document is necessary for reporting purposes. The Medical Genomics Laboratory issues its clinical reports based on the demographic data provided by the referring institution on the lab requisition form. It is the responsibility of the referring institution to provide accurate information. If an amended report is necessary due to inaccurate or illegible documentation, additional reports will be drafted with charge.

Requests for testing may not be accepted for the following reasons:

- No label (patients full name and date of collection) on the specimens
- No referring physician's or genetic counselor's names and addresses
- No billing information
- DNA samples must be extracted in a CLIA or equivalent certified lab

For more information, test requisition forms, or sample collection and mailing kits, please call: 205-934-5562.

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Disorder Background

Germline loss-of-function variants in *SPRED1*, a negative regulator of the RAS-MAPK pathway, cause a neurofibromatosis type 1-like phenotype, first described in 2007 (Legius syndrome). Patients present with multiple café-au-lait spots with or without skinfold freckling. Other typical NF1 associated features (Lisch nodules, bone abnormalities, neurofibromas, optic pathway gliomas) are systematically absent. However, in some individuals Noonan-like features have been reported.

In individuals with CALMs with or without freckling and no other specific distinguishing features, the NIH criteria cannot reliably distinguish NF1 from Legius syndrome. In such patients, a correct diagnosis has important implications for prognosis, counseling, and potential prenatal genetic diagnosis. Based on a cross-sectional study we estimate that patients presenting sporadically with these pigmentary signs only will carry a variant in the *NF1* gene in ~43% of cases and in the *SPRED1* gene in ~1.3% of cases. When such patients have a family history of CALMs with or without freckling and no additional NF1-related criteria, an *NF1* variant will be found in ~73% of cases and in the *SPRED1* gene in ~19% of cases. *SPRED1* is a member of the SPROUTY/SPRED family of proteins that act as negative regulators of RAS-RAF interaction and mitogen-activated protein kinase (MAPK) signaling.

Test Description

The **RNA-based *NF1* and DNA-based *SPRED1* testing** on blood requires a **fresh EDTA blood** sample, to arrive in the lab <60-70 hours after blood draw. **DNA** is extracted and in addition, a short term phytohemagglutinin-stimulated lymphocyte culture is initiated and used as starting material to extract **RNA**. The complete *NF1* coding region is analyzed by a cascade of complementary variant detection techniques, including RT-PCR, direct sequencing of cDNA fragments, microsatellite marker analysis and copy number analysis by MLPA, enabling identification of the variant in ~95% of non-founder patients fulfilling the NIH diagnostic criteria.

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RNA-based *NF1* testing allows finding deep intronic splice variants through their observed effect on splicing. During the >15 years we have offered comprehensive RNA-based *NF1* testing on blood, we have identified >65 different locations harboring deep intronic splice variants: together they account for 2.5% of all pathogenic variants identified in the *NF1* UAB cohort. Please note that all known deep intronic splice variants have been incorporated in the *customized* UAB NGS available assays.

In addition, all coding exons and flanking intronic sequences of the *SPRED1* gene are analyzed by bidirectional sequencing and deletion/duplication analysis is performed using MLPA.

REFERENCES available on website.

Other related testing options:

- Next-Gen Sequencing and Deletion/Duplication analysis of *NF1* only (NF1-NG)
- Next-Gen Sequencing and Deletion/Duplication analysis of *NF1* and *SPRED1* only (NFSP-NG)
- Expanded *NF1*-Rasopathy panel by Next-Gen Sequencing (RAS-NG)
- RNA-based *NF1/SPRED1* testing on affected tissues (NF14N/NF14C)

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