UAB MEDICAL GENOMICS LABORATORY

RNA-based *NF1* Testing on Blood (NF1-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:

$1,800 (USD – institutional/self-pay);

CPT: 88230, 81408, and 81479

Z code: ZB6AF

Candidates for this test:

1)Patients who need the most sensitive and specific test with the fastest turnaround time (e.g. patients with an ongoing pregnancy); and 2) non- founder patients a) with a clear cut clinically documented classic NF1, b) from a clinically documented multi-generation (minimum 3 generations) family, c) who tested negative by the MGL NF1-only NGS assay and d) in whom a translocation has been excluded by cytogenetic analysis. The latter patients will receive free of charge reflex RNA-based NF1 testing, which should allow to detect a possible deep intronic splice variant not previously identified in the UAB cohort and not reported elsewhere, or a possible Alu/LINE insertion or other exotic complex variant.

Specimen shipping and handling:

720 Twentieth Street South, Suite 330 Phone: (205) 934-5562
Birmingham, Alabama 35294-0005 Fax: (205) 996-2929
www.uab.edu/medicine/genetics/medical-genomics-laboratory

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- 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.
- 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

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- 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.

Disorder Background

The NF1 gene, cloned in 1990, was the first gene within the Ras-MAPK pathway shown to be associated with an autosomal dominant disorder, Neurofibromatosis type I (NF1). NF1 affects ~1/3000 individuals worldwide, with half of the patients being sporadic. NF1 is notorious for its phenotypic variability and is a progressive disorder with more signs developing with time. Although the NIH criteria enables clinicians to make a diagnosis in the majority of classically affected cases, diagnostic criteria are not met until a given age is reached. Atypical presentations also exist with patients not yet fulfilling NIH criteria by adulthood. The variant spectrum of NF1 is very complex and includes a wealth of unusual splice variants affecting exonic sequences as well as deep intronic variants resulting in exonization of intronic sequences at the mRNA level.

Test Description

The **RNA-based *NF1* 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.
RNA-based *NF1* testing allows finding deep intronic splice variants through their observed

effect on splicing. These splice variants would not be detected if a "simple" exon-by-exon DNA-based (NGS/Sanger) sequencing approach is used. 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.

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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