UAB MEDICAL GENOMICS LABORATORY

RNA-based *NF1*/*SPRED1* Testing on Cultured from Affected Tissues (NF14N/NF14C)

Ordering Information

Acceptable specimen types:

- A minimum of 2, 2-3mm anatomically distinct café-au-lait spot punch biopsies submitted in our provided culture media. Call, email, or see website to request a media collection kit
- A minimum of 2 anatomically distinct fresh neurofibroma biopsies submitted in our provided culture media. Call, email, or see website to request a media collection kit
- A blood (3-6ml whole blood in EDTA) specimen may be provided for targeted testing of any suspected germline (1st hit mutations) identified during biopsy based testing

Turnaround time:

6 months

Price, CPT codes, and Z code:

$2,600 for *NF1*-only* (USD – institutional/self-pay);

CPT: 88233, 81408 and 81479

Z code: ZB6AG for CALs, ZB67X for neurofibromas

*When necessary, with added reflex to *SPRED1* testing: $3,200 (USD – institutional/self-pay); additional CPT codes 81405 and 81404

Candidates for this test:

Patients suspected to have segmental NF1, with symptoms restricted to a defined area of the body; sporadic patients who have (mild) non-localized symptoms of NF1 and in whom

no *NF1* mutation was identified in the blood lymphocytes and may have disease due to a postzygotic mutation; reflex testing for familial or sporadic patients with a first hit mutation refractory to detection by RNA- or DNA/NGS assay

Specimen shipping and handling:

- Please find acceptable specimen type above.
- Contact us to request a media collection kit or set up a time to discuss your patient *prior* to taking biopsy/biopsies in your patient, so we can provide individualized advice and ship out appropriate collection/transport media and forms prior to the procedure
- Please see website or contact us for instructions for collecting and shipping skin biopsies (CAL-spots) or neurofibromas for NF1/SPRED1 testing
- 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).
- 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.

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 mutational spectrum of NF1 is very complex and includes a wealth of unusual splice mutations affecting exonic sequences as well as deep intronic mutations resulting in exonization of intronic sequences at the mRNA level.

Some individuals with a clinical diagnosis of one of the RASopathies have been found to carry a mutation in a gene that was not considered to be consistent with their clinical diagnosis. Examples include *BRAF* variants reported in individuals with a clinical diagnosis of Noonan syndrome, a *SOS1* variant in an individual with CFC (Nystrom AM et al, 2008), *PTPN11* mutations in individuals with paraspinal neurofibromas (Conboy E. et al, 2015), and an *NF1* missense mutation in patients with Noonan-like features and no neurofibromas (Rojnueangnit K et al, 2015). In addition, some genes are associated with more than one syndrome (*PTPN11, KRAS, BRAF, RAF1*, and *NF1*). Therefore, the comprehensive approach of simultaneously testing all 16 genes in some individuals eliminates the need to determine which genes to test based on an individual's clinical signs.

Test Description

The **RNA-based NF1/SPRED1 testing on cultured cells from affected tissu**es is offered starting from **biopsies** of café-au-lait macules (CALM) and/or neurofibromas. Melanocytes cultured from CALMs and Schwann cells cultured from neurofibromas are the starting material to extract RNA. The complete *NF1* coding region is analyzed by a cascade of complementary mutation detection techniques, including RT-PCR, direct sequencing of cDNA fragments, microsatellite marker analysis, copy number analysis by MLPA and interphase FISH (if a total gene deletion is detected by copy number analysis), enabling identification of the mutation in ~95% of non-founder patients fulfilling the NIH diagnostic criteria.

RNA-based *NF1* testing allows finding deep intronic splice mutations through their observed effect on splicing. These splice mutations 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, we have identified >65 different locations harboring deep intronic splice mutations; together they account for 2.5% of all pathogenic mutations identified in the *NF1* UAB cohort. Please note that all known deep intronic splice mutations

have been incorporated in the customized UAB NGS available assays.

In addition, for patients with only pigmentary features (CALMs with/without skinfold freckling but no neurofibromas), and no *NF1* mutations found in the melanocytes (no first or second hit mutations), the *SPRED1* gene is analyzed as a reflex testing free of charge (sequencing and deletion/duplication analysis), as these patients may have mosaic Legius syndrome. As a result of this test, if features are *NF1* or *SPRED1*-related, a common first hit is identified in both biopsies and a different second hit is identified in every anatomically different biopsy evaluated. If no mutations are identified despite full analysis on 2 biopsies with successful cultures, (mosaic) NF1/Legius syndrome is very unlikely (<0.2%).

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