X-linked Adrenoleukodystrophy: *ABCD1* Gene Sequencing

**Condition Description**

X-linked adrenoleukodystrophy (X-ALD) is a disorder of fatty acid oxidation cause by mutations in the *ABCD1* gene (Xq28). X-ALD can present at a variety of ages and with different manifestations depending on the presence and type of neurologic findings. The condition has three main phenotypes seen in males. In the childhood cerebral form, symptoms appear between ages four and eight years and include inattention, hyperactivity, and emotional lability. Progressive impairment of cognition, behavior, vision, hearing, and motor function follow the initial symptoms and often lead to total disability within two years. The second phenotype, adrenomyeloneuropathy (AMN), manifests most commonly in the late twenties as progressive paraparesis, sphincter disturbances, sexual dysfunction, and often, impaired adrenocortical function; all symptoms are progressive over decades. The third phenotype, “Addison disease only,” presents with primary adrenocortical insufficiency between age two years and adulthood (most commonly by age 7.5 years), without evidence of neurologic abnormality. Some degree of neurologic disability (most commonly AMN), however, usually develops later. Varying phenotypes often coexist in the same kindred or sibship. Approximately 20% of carrier females develop neurologic manifestations that resemble adrenomyeloneuropathy, but have later onset (age 35 years or later) and milder disease than affected males.

The *ABCD1* gene encodes the ATP-binding cassette sub-family D member 1 protein, which is located in the peroxisomal membrane. Gene product is absent in 70% of affected individuals. The principal biochemical abnormality is the accumulation of saturated very long chain fatty acids (VLCFA) due to an apparent defect in peroxisomal beta oxidation, particularly hexacosanoic (C26:0) and tetracosanoic (C24:0) fatty acid, a function that normally takes place in the peroxisome. Testing for plasma concentration of VLCFA reveals abnormal levels in 99.9% of males with X-ALD. Increased concentration of VLCFA in plasma and/or cultured skin fibroblasts is present in approximately 85% of affected females; 20% of known carriers have normal plasma concentration of VLCFA.

About 93% of index cases have inherited the *ABCD1* mutation from one parent; at most, 7% of individuals with X-ALD have *de novo* mutations. PCR and sequence analysis identified mutations in 229 of 249 (92%) hemizygous males or obligate heterozygote females. Sixteen of the 20 individuals (in the series of 249 individuals) without a mutation identified by sequence analysis had a deletion detected by Southern blot analysis. In one of the four remaining individuals, Southern blot results suggested a duplication or rearrangement, for a total detection rate of 98%. The prevalence is estimated to be between 1:20,000 and 1:50,000. The minimum frequency of hemizygotes identified in the United States is estimated to be 1:21,000 and that of hemizygotes plus heterozygotes (i.e., carrier females) is 1:16,800. The prevalence appears to be approximately the same in all ethnic groups.

For patients with suspected X-ALD, sequence analysis is recommended as the first step in mutation identification. For patients in whom mutations are not identified by full gene sequencing, deletion/duplication analysis is appropriate.

**Genes**

*ABCD1*

**Indications**

- Confirmation of a clinical/biochemical diagnosis of X-ALD
- Carrier testing in adult females with a family history of X-ALD

**Methodology**

PCR amplification of 10 exons contained in the *ABCD1* gene is performed on the patient's genomic DNA. Direct sequencing of amplification products is performed in both forward and reverse directions, using automated fluorescence dyeoxy sequencing methods. The patient's gene sequences are then compared to a normal reference sequence. Sequence variations are classified as mutations, benign variants unrelated to disease, or variations of unknown clinical significance. Variants of unknown clinical significance may require further studies of the patient and/or family members. This assay does not interrogate the promoter region, deep intronic regions, or other regulatory elements, and does not detect large deletions.

**Detection**

Clinical Sensitivity: Sequence analysis identified mutations in 229 of 249 (92%) hemizygous males or obligate heterozygote females. Mutations in the promoter region, some mutations in the introns and other regulatory element mutations cannot be detected by this analysis. Large deletions will not be detected by this analysis. Results of molecular analysis should be interpreted in the context of the patient's biochemical phenotype.

Analytical Sensitivity: ~99%

**Specimen Requirements**

Submit only 1 of the following specimen types

* Preferred specimen type: Whole Blood

**Type:** Whole Blood
Specimen Requirements:

In EDTA (purple top) tube:
Infants (2 years): 3-5 ml
Older Children & Adults: 5-10 ml

Specimen Collection and Shipping: Refrigerate until time of shipment. Ship sample within 5 days of collection at room temperature with overnight delivery.

**Type: Saliva**

Specimen Requirements:

Oragene™ Saliva Collection kit (available through EGL) used according to manufacturer instructions.

Specimen Collection and Shipping: Store sample at room temperature. Ship sample within 5 days of collection at room temperature with overnight delivery.

**Special Instructions**

Submit copies of diagnostic biochemical test results with the sample, if appropriate. Contact the laboratory if further information is needed.

**Related Tests**

- Deletion/duplication analysis of the *ABCD1* gene by CGH array is available for those individuals in whom sequence analysis is negative.
- A CGH array-based test for deletion/duplication analysis of 64 different X-linked intellectual disability genes is available (OL).
- **Custom diagnostic mutation analysis (KM)** is available to family members if mutations are identified by targeted mutation testing or sequencing analysis.
- Prenatal testing is available to adult females who are confirmed carriers of mutations. Please contact the laboratory genetic counselor to discuss appropriate testing prior to collecting a prenatal specimen.