FirmaLab™ Bio-Diagnostics

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Patient Name: **John Doe**

DOB	Gender	Ethnicity
05/07/2014	M	Unknown

Test Ordered	Test Result	Expected (Negative) Value
LAL (LIPA) Enzyme Assay Dry Blood Spot	**Negative (0.29-2.30 nmole/punch/hour)**	Negative (0.29-2.30 nmole/punch/hour)

Result Note: 0.41 nmole/punch/hour. Case discussed with treating physician. The normal (negative) range reported may not reflect age/sex matched controls. Based on DNA sequence results (below) and clinical presentation, it is possible that the promoter region or splicing may result in lower than expected LAL enzyme expression and activity. Below automated Result Interpretations are based on established normal range.

Result Interpretation: LAL enzyme activity is within normal reference range, which indicates normal LAL enzyme activity. This result is not consistent with the diagnosis of LAL deficiency.

Method of Analysis: The measurement of lysosomal acid lipase (LAL) in dried blood spots (DBS) is done using a fluorimetric substrate, 4-methylumbelliferyl palmitate (4mU palmitate), with cardiolipin present as an activator of LAL. The presence of other forms of lipase in whole blood will interfere with the measurement of LAL. Since Lalistat 2 is a specific inhibitor of LAL, measuring the total lipase activity and lipase activity in the presence of Lalistat 2 will allow for determination of LAL in DBS. If an individual has been the recipient of bone marrow transplantation, or if he/she has undergone any blood transfusion procedure within the past six months, blood may not be tested reliably. This test was developed and its performance characteristics determined by the laboratory. The principle of the assay was based on studies described by Hamilton J, et al, Clinica Chimica Acta (2012), doi:10.1016/j.cca.2012.03.019.

The assay has not been cleared or approved by the U.S. Food and Drug Administration (FDA), or the FDA has determined that such clearance of approval is not necessary. The laboratory is accredited by College of American Pathologists (CAP), and regulated under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity molecular testing.

LIPA Sequence (Wolman Disease)	***Variations found (Unknown significance)***	Negative (No variations found)

Result Note: 18 different variations and SNP's found (see attached for complete list). 4 of these variations have not been reported as SNP's, of which one is in the promoter region, and two are within 535 - 1004 bases to the start codon. It is possible that These variations may either affect the expression levels or splicing of LAL enzyme. Below automated Result Interpretations are based on established normal range.

Result Interpretation: Variation found in DNA region that is unlikely to result in disease process (e.g. Single Nucleotide Polymorphism listed in dbSNP, silent mutation without translational change in protein amino acid sequence, non-coding base change of an evolutionary unconserved region, etc.). Amino acid numbering is based on GenBank NM_001127605. See additional information attached.

Electronically signed by: Daniel Darvish, MD, on 02/10/2015 12:38
Report Type: Complete

Specimen ID	Specimen Type	Collection Date Time	Date Received	Report Date
1001007	Whole Blood (EDTA)		01/30/2015 10:00	02/09/2015 17:24

Laboratory Director: Marvin Pietruszka, MD, JD
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Test Requested: Sequencing of the *LIPA* gene as genetic confirmation of Liposomal Acid Lipase deficiency (Wolman Disease, WD, LAL deficiency, or Cholesteryl Ester Storage Disease, CESD) or carrier testing for high risk individuals (e.g. family members).

Appropriate genetic counseling should be utilized to explain the implications of the test result, its residual risks and uncertainties. Although DNA-based testing is highly accurate, rare diagnostic errors may occur. Examples include misinterpretation because of genetic variants, blood transfusion, bone marrow transplantation, gene therapy, cell therapy, erroneous representation of family relationships, or contamination of a fetal sample with maternal cells.

WD is an autosomal recessive disorder. The results are characterized as heterozygous, or homozygous for each DNA variation found. A negative result from this analysis cannot eliminate the possibility that an individual carries a non-coding mutation that may affect the expression or splicing of the gene. The disease penetrance of homozygous individuals is unknown. Carrier or heterozygous individuals are unlikely to become afflicted with disease phenotype.

WD is severe rare infantile form of inherited lysosomal lipid storage diseases due to deficiency of acid lipase (sterol esterase), and it is characterized by the accumulation of neutral lipids, particularly cholesterol esters in leukocytes, fibroblasts, and hepatocytes. WD is an allelic variant of Cholesterol Ester Storage Disease (CESD). In CESD at least one mutant allele produces limited enzymatic function and improves the phenotype, likely leading to the more benign clinical course of the disease (Anderson & Sando 1991). In contrast to CESD, mutations that lead to WD cause a more dramatic reduction of enzyme activity. There is no single defining mutation for WD, and may vary based on genetic background of the population. For example, G87V is the most common variation that leads to WD in infants born to Iranian-Jewish parents. The carrier frequency of G87V in people of Iranian-Jewish background is estimated at 1:55 or 1.82% (Valles-Ayoub et al. 2011).

References

Anderson, R.A. & Sando, G.N., 1991. Cloning and expression of cDNA encoding human lysosomal acid lipase/cholesteryl ester hydrolase. Similarities to gastric and lingual lipases. *The Journal of Biological Chemistry*, 266(33), pp.22479–22484. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1718995 [Accessed December 24, 2010].

Valles-Ayoub, Y. et al., 2011. Wolman Disease (LIPA p.G87V) Genotype Frequency in People of Iranian-Jewish Ancestry. *Genetic Testing and Molecular Biomarkers*. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21291321 [Accessed February 5, 2011].

Method of Analysis:

Genomic DNA is isolated from the sample and exons of the gene are amplified enzymatically and analyzed using automated sequencing. DNA segments are tested by multiple forward and reverse sequence runs to obtain a consensus sequence. The readability of each sequence result is ranked and poorly readable or questionable sequences are repeated. The resulting sequence reads are blasted against human *LIPA* genomic sequence (GenBank accession NG_008194). Multiple sequence runs of the same DNA strand are used to confirm positive or negative findings.

This test was developed and its performance characteristics determined by the laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA), or the FDA has determined that such clearance of approval is not necessary. The laboratory is accredited by College of American Pathologists (CAP), and certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity molecular testing.