Client: Example Client ABC123
123 Test Drive
Salt Lake City, UT 84108
UNITED STATES

Physician: Doctor, Example

Patient: Patient, Example

DOB	4/14/1945
Gender:	Female
Patient Identifiers:	01234567890ABCD, 012345
Visit Number (FIN):	01234567890ABCD
Collection Date:	00/00/0000 00:00

Peroxisomal Disorder Panel, Sequencing

ARUP test code 3002700

Peroxisomal Disorders Specimen	Whole Blood
Peroxisomal Disorders Interp	Positive

RESULT
Two apparent copies of a pathogenic variant were detected in the AGXT gene.

PATHOGENIC VARIANT
Gene: AGXT (NM_000030.3)
Nucleic Acid Change: c.508G>A; Homozygous
Amino Acid Alteration: p.Gly170Arg
Inheritance: Autosomal recessive

INTERPRETATION
Two apparent copies of a pathogenic variant, c.508G>A; p.Gly170Arg, were detected in the AGXT gene by massively parallel sequencing. Pathogenic variants in AGXT are inherited in an autosomal recessive manner and are associated with primary type 1 hyperoxaluria (MIM: 259900). Although copy number cannot be determined by this assay, large deletions/duplications are rare in the AGXT gene; therefore, this result may represent homozygosity for the identified variant or a single copy of the variant with a large deletion on the opposite chromosome. Parental testing could determine which of the above scenarios is correct for the purpose of testing other family members. This individual is predicted to be affected with primary type 1 hyperoxaluria if the two identified variants are on opposite chromosomes; clinical manifestations are variable.

Please refer to the background information included in this report for a list of the genes analyzed, methodology, and limitations of this test.

Evidence for variant classification:
The AGXT c.508G>A; p.Gly170Arg variant (rs121908529) is reported in the literature in compound heterozygous and homozygous individuals affected with primary type 1 hyperoxaluria (PH1; Coulter-Mackie 2001, Purdue 1990, Monico 2005) and accounts for one third of PH1-causing alleles (Milliner 2002). This variant is also reported in ClinVar (Variation ID: 40166) and is found in the general population with an overall allele frequency of 0.06% (136/242,084 alleles, including 1 homozygote) in the Genome Aggregation Database (v2.1.1). Computational analyses predict that this variant is deleterious (REVEL: 0.913) and functional analyses demonstrate when found in cis with benign polymorphism, p.Pro11Leu, results in mistargeting of the AGT enzyme to the mitochondria rather than the peroxisomes (Coulter-Mackie 2005, Lumb 1999, Lumb 2000). Based on available

information, the p.Gly170Arg variant is considered to be pathogenic.

RECOMMENDATIONS
Genetic consultation is recommended, including a discussion of medical screening and management. At-risk family members should be offered testing for the identified pathogenic AGXT variant (Familial Targeted Sequencing, ARUP test code 3005867). Deletion/duplication analysis of AGXT or parental testing for the identified variant is recommended to exclude the rare possibility that this individual actually has one copy of the identified variant on one chromosome and a deletion on the other. This individual's reproductive partner should be offered genetic testing to determine carrier status.

BENIGN VARIANT
AGXT (NM_000030.3) c.32C>T; p.Pro11Leu - Homozygous
The AGXT c.32C>T; p.Pro11Leu variant (rs34116584) is listed in the ClinVar database (Variation ID: 5641) and is observed in the general population with an overall frequency of 14.8% (41,051/247,024 alleles, including 3886 homozygotes) in the Genome Aggregation Database (v2.1.1). The p.Pro11Leu is a benign polymorphism that is associated with the AGXT minor allele haplotype. The minor allele does not cause PH1 by itself but is known to exacerbate the deleterious effects of other pathogenic variants when found in cis (Milliner 2002). Based on available information, this variant is considered to be benign.

COMMENTS
Unless otherwise specified, confirmation by Sanger sequencing was not performed for variants with acceptable quality metrics. All other likely benign and benign variants are not reported. Variants in the following region(s) may not be detected by NGS with sufficient confidence in this sample due to technical limitations: None

REFERENCES
Coulter-Mackie MB, Rumsby G, Applegarth DA, Toone JR. Three novel deletions in the alanine:glyoxylate aminotransferase gene of three patients with type 1 hyperoxaluria. Mol Genet Metab. 2001 Nov;74(3):314-21. doi: 10.1006/mgme.2001.3222. PMID: 11708860.
Coulter-Mackie MB et al. Overexpression of human alanine:glyoxylate aminotransferase in Escherichia coli: renaturation from guanidine-HCl and affinity for pyridoxal phosphate co-factor. Protein Expr Purif. 2005 May;41(1):18-26. PMID: 15802217.
Lumb MJ et al. Effect of N-terminal alpha-helix formation on the dimerization and intracellular targeting of alanine:glyoxylate aminotransferase. J Biol Chem. 1999 Jul 16;274(29):20587-96. PMID: 10400689.
Lumb MJ et al. Functional synergism between the most common polymorphism in human alanine:glyoxylate aminotransferase and four of the most common disease-causing mutations. J Biol Chem. 2000 Nov 17;275(46):36415-22. PMID: 10960483.
Milliner DS, et al. Primary Hyperoxaluria Type 1. 2002 Jun 19 [updated 2022 Feb 10]. In: Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, editors. GeneReviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. PMID: 20301460.
Monico CG et al. Pyridoxine effect in type I primary hyperoxaluria is associated with the most common mutant allele. Kidney Int. 2005 May;67(5):1704-9. PMID: 15840016.
Purdue PE et al. Identification of mutations associated with peroxisome-to-mitochondrion mistargeting of alanine/glyoxylate aminotransferase in primary hyperoxaluria type 1. J Cell Biol. 1990 Dec;111(6 Pt 1):2341-51. PMID: 1703535.

This result has been reviewed and approved by [redacted]

BACKGROUND INFORMATION: Peroxisomal Disorders Panel, Sequencing

H=High, L=Low, *=Abnormal, C=Critical

CHARACTERISTICS: Peroxisomal disorders are a group of diseases caused by gene defects impairing the formation (peroxisome biogenesis disorders) or function of the peroxisomes, with symptoms that impact a wide range of body systems. Peroxisome biogenesis disorders include Zellweger spectrum disorders (ZSD) and rhizomelic chondrodysplasia punctata (RCDP). Single enzyme defects include Refsum disease, peroxisomal acyl-CoA oxidase deficiency, peroxisomal bifunctional deficiency, defects of bile acid synthesis, and primary hyperoxaluria. Some single enzyme defects present with similar clinical features to ZSD (e.g. ACOX1, HSD17B4) or RCDP (e.g. AGPS, GNPAT), although these often can be distinguished by extensive biochemical testing. Signs and symptoms of peroxisomal disorders may develop as early as the newborn period, with hypotonia, seizures, poor growth, and feeding problems. Leukodystrophy, hepatic dysfunction, adrenal insufficiency, hearing loss, and visual impairment may also be present. Skeletal abnormalities in individuals with peroxisomal disorders include stippling of the growth plates and chondrodysplasia punctata, or progressive loss of bone mineral density. Later onset forms of these conditions have similar symptoms, but with a slower progression and milder severity. Developmental delay and intellectual disability are common.

INCIDENCE: Approximately 1 in 50,000

CAUSE: Pathogenic germline variants in genes associated with the structure and function of peroxisomes.

INHERITANCE: Autosomal recessive with rare autosomal dominant cases

CLINICAL SENSITIVITY: At least 97% for Zellweger spectrum disorders
At least 97% for rhizomelic chondrodysplasia punctata

GENES TESTED: ABCD3, ACBD5*, ACOX1, AGPS, AGXT, AMACR, DNM1L, FAR1, GNPAT, HSD17B4, PEX1, PEX10, PEX11B, PEX12, PEX13, PEX14, PEX16, PEX19, PEX2, PEX26, PEX3, PEX5, PEX6, PEX7, PHYH, SCP2*

* One or more exons are not covered by sequencing for the indicated gene; see limitations section below.

METHODOLOGY: Capture of all coding exons and exon-intron junctions of the targeted genes, followed by massively parallel sequencing. Sanger sequencing was performed as necessary to fill in regions of low coverage and confirm reported variants.

ANALYTICAL SENSITIVITY/SPECIFICITY: The analytical sensitivity of this test is approximately 99 percent for single nucleotide variants (SNVs) and greater than 93 percent for insertions/duplications/deletions from 1-10 base pairs in size. Variants greater than 10 base pairs may be detected, but the analytical sensitivity may be reduced.

LIMITATIONS: A negative result does not exclude a diagnosis of peroxisomal disorders. This test only detects variants within the coding regions and intron-exon boundaries of the targeted genes. Regulatory region variants and deep intronic variants will not be identified. Deletions/duplications/insertions of any size may not be detected by massively parallel sequencing. Diagnostic errors can occur due to rare sequence variations. In some cases, variants may not be identified due to technical limitations in the presence of pseudogenes, repetitive, or homologous regions. This assay may not detect low-level mosaic or somatic variants associated with disease. Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation. Non coding transcripts were not analyzed.

The following regions are not sequenced due to technical limitations of the assay:

ACBD5 (NM_001352568) exon(s) 6
ACBD5 (NM_001352569) exon(s) 6
ACBD5 (NM_001352570) exon(s) 13
ACBD5 (NM_001352571) exon(s) 5
ACBD5 (NM_001352573) exon(s) 6
ACBD5 (NM_001352574) exon(s) 6
ACBD5 (NM_001352575) exon(s) 6
ACBD5 (NM_001352576) exon(s) 6
ACBD5 (NM_001352581) exon(s) 6
ACBD5 (NM_001352585) exon(s) 5
ACBD5 (NM_001352586) exon(s) 5
ACBD5 (NM_001352568) partial exon(s) 1(Chr10:27529638-27529648)
ACBD5 (NM_001352572) partial exon(s) 1(Chr10:27529638-27529648)
SCP2 (NM_001007098) exon(s) 11
SCP2 (NM_001330587) exon(s) 12

This test was developed and its performance characteristics determined by ARUP Laboratories. It has not been cleared or approved by the US Food and Drug Administration. This test was performed in a CLIA certified laboratory and is intended for clinical purposes.

Counseling and informed consent are recommended for genetic testing. Consent forms are available online.

VERIFIED/REPORTED DATES

Procedure	Accession	Collected	Received	Verified/Reported
Peroxisomal Disorders Specimen	23-363-100623	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Peroxisomal Disorders Interp	23-363-100623	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00

END OF CHART

H=High, L=Low, *=Abnormal, C=Critical