ARUP LABORATORIES
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Jonathan R. Genzen, MD, PhD, Chief Medical Officer

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

Physician: Doctor, Example

Patient: Patient, Example

DOB	7/11/2002
Gender:	Female
Patient Identifiers:	01234567890ABCD, 012345
Visit Number (FIN):	01234567890ABCD
Collection Date:	00/00/0000 00:00

Hemoglobin Evaluation Reflexive Cascade

ARUP test code 2005792

Hemoglobin A	**65.2 % L**	**(Ref Interval: 95.0-97.9)**
Hemoglobin A2	2.7 %	(Ref Interval: 2.0-3.5)
Hemoglobin F	0.6 %	(Ref Interval: 0.0-2.1)
	REFERENCE INTERVAL: Hemoglobin F Access complete set of age- and/or gender-specific reference intervals for this test in the ARUP Laboratory Test Directory (aruplab.com).	
Hemoglobin S	**31.5 % H**	**(Ref Interval: 0.0-0.0)**
Hemoglobin C	0.0 %	(Ref Interval: 0.0-0.0)
Hemoglobin E	0.0 %	(Ref Interval: 0.0-0.0)
Hemoglobin - Other	0.0 %	(Ref Interval: 0.0-0.0)
Sickle Cell Solubility	Conf Previous	
Hemoglobin, Capillary Electrophoresis	Performed	
Hemoglobin Evaluation	See Note	
Beta Globin Full Gene Sequencing	Performed	

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

Beta Globin (HBB) Del/Dup Result	Not Applicable
Alpha Thalassemia HBA1 and HBA2 Seq	Not Applicable
Gamma Globin (HBG1 and HBG2) Sequencing	Not Applicable
Hemoglobin Cascade Interpretation	See Note

RESULT
One copy of the Hb S pathogenic variant was detected in the beta globin (HBB) gene.
Alpha (+) thalassemia (silent carrier) state (-a/aa).
See comments.

COMMENTS
One copy of the Hb S pathogenic variant was detected in the beta globin (HBB) gene by massively parallel sequencing, consistent with sickle cell trait. The clinical presentation may vary due to other genetic modifiers or co-existing conditions.

One copy of the 3.7 Kb alpha globin gene deletion was detected by deletion/duplication analysis of the alpha globin gene cluster and its HS-40 regulatory region. This result is consistent with the deletion of a single alpha gene and predicts alpha (+) thalassemia (silent carrier). Heterozygosity for the 3.7 Kb deletion does not result in clinical symptoms but may lead to erroneous diagnosis of and treatment for iron deficiency. The variant is common among African Americans.

Please refer to the background information included in this report for the clinical sensitivity and limitations of this test.

Minor components of Hb S and other hemoglobin variants eluting after HbA2 may co-elute with HbA2. This may result in a falsely elevated value for HbA2.

DNA MUTATIONS/VARIANTS
1. PATHOGENIC VARIANT
Gene: HBB (NM_000518.5)
Nucleic Acid Change: c.20A>T; Heterozygous
Amino Acid Alteration: p.Glu7Val
Commonly Known As: Hb S
Inheritance: Autosomal recessive

2. Pathogenic Deletion: -alpha3.7; Heterozygous
Predicted Genotype: -a/aa

Evidence for variant classification:
The Hb S variant (HBB: c.20A>T; p.Glu7Val, also known as Glu6Val when numbered from the mature protein, HbVar ID: 226, rs334) is a common pathogenic beta globin variant. Heterozygosity for Hb S is consistent with sickle cell trait. Homozygosity for Hb S results in sickle cell anemia. Hb S in combination with a different pathogenic HBB variant on the opposite chromosome results in various forms of sickle cell disease (see HbVar link and references therein).

RECOMMENDATIONS
Medical management should rely on clinical findings and family history. Family members should be offered carrier testing for the identified variant. This individual's reproductive partner

should be offered carrier testing for hemoglobinopathies. Genetic consultation is recommended.

REFERENCES
Link to HbVar database: https://globin.bx.psu.edu/hbvar/menu.html

NOTES
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; reportable variants are confirmed by Sanger sequencing:
NONE

Reference sequence for alpha globin gene cluster: NG_000006.1

Controls were run and performed as expected.
This result has been reviewed and approved by [redacted]

BACKGROUND INFORMATION: Beta Globin (HBB) Sequencing

CHARACTERISTICS: Beta thalassemia is caused by decreased or absent synthesis of the hemoglobin beta-chain resulting in variable clinical presentations ranging from mild anemia to transfusion dependence. Structural hemoglobinopathies may result in sickling disorders, microcytic or hemolytic anemia, cyanosis, or erythrocytosis.

EPIDEMIOLOGY: Incidence varies by ethnicity.

CAUSE: Pathogenic germline variants within the HBB gene.

INHERITANCE: Usually autosomal recessive, infrequently autosomal dominant.

CLINICAL SENSITIVITY: Up to 99 percent, depending upon ethnicity, for beta thalassemia and hemoglobinopathies associated with the HBB gene.

GENE TESTED: HBB (NM_000518)
Deletion/duplication analysis is not available for this gene.

METHODOLOGY: Probe hybridization-based capture of all coding exons, exon-intron junctions, 5' proximal promoter and untranslated region, 3' polyadenylation signal, and intronic variants c.93-21G>A (IVS-I-110), c.316-197C>T (IVS-II-654), c.316-146T>G (IVS-II-705), and c.316-106C>G (IVS-II-745) of the HBB gene, followed by massively parallel sequencing. Sanger sequencing was performed as necessary to fill in regions of low coverage and to confirm reported variants that do not meet acceptable quality metrics.

ANALYTICAL SENSITIVITY/SPECIFICITY: The analytical sensitivity is approximately 99 percent for single nucleotide variants (SNVs) and greater than 93 percent for insertions/duplications/deletions (indels) from 1-10 base pairs in size. Indels 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 beta thalassemia. This test detects variants within the coding regions and intron-exon boundaries of the HBB gene. Deletions/duplications/insertions of any size may not be detected by massively parallel sequencing. Regulatory region variants upstream of c.-250, deep intronic variants (other than those described in methodology section above), and large deletions/duplications will not be identified. Diagnostic errors can occur due to rare sequence variations. In some cases, variants may not be identified due to technical limitations caused by the presence of pseudogenes, repetitive, or homologous regions. This test is not intended to detect low-level mosaic or somatic variants, gene conversion events, complex inversions,

translocations, mitochondrial DNA (mtDNA) variants, or repeat expansions. Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation. Noncoding transcripts were not analyzed.

This test was developed and its performance characteristics determined by ARUP Laboratories. It has not been cleared or approved by the U.S. 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.

BACKGROUND INFORMATION: Alpha Globin (HBA1 and HBA2) Deletion/Duplication
CHARACTERISTICS: Alpha thalassemia is caused by decreased or absent synthesis of the hemoglobin alpha-chain resulting in variable clinical presentations. Alpha (+) thalassemia results from mutation of a single alpha2 globin gene (-a/aa) and is clinically asymptomatic (silent carrier). Alpha (0) thalassemia (trait) is caused by mutation of both alpha2 globin genes (-a/-a), or mutations in the alpha1 and alpha2 globin genes on the same chromosome, (--/aa) and results in mild microcytic anemia. Hemoglobin H disease occurs due to mutation of three alpha globin genes (--/-a) and results in hemolysis with Heinz bodies, moderate anemia, and splenomegaly. Hb Bart Hydrops Fetalis Syndrome results when mutations occur in all four alpha globin genes (--/--) and is lethal in the fetal or early neonatal period. Alpha globin gene triplications result in three active alpha globin genes on a single chromosome.
INCIDENCE: Carrier frequency in Mediterranean (1:30-50), Middle Eastern, Southeast Asian (1:20), African, African-American (1:3).
INHERITANCE: Autosomal recessive.
CAUSE: Pathogenic mutations in the alpha globin gene cluster.
CLINICAL SENSITIVITY: Varies by ethnicity, up to 95 percent.
METHODOLOGY: Multiplex ligation-dependent probe amplification (MLPA) of the alpha globin gene cluster (HBZ, HBM, HBA2, HBA1, HBQ1) and its HS-40 regulatory region.
ANALYTICAL SENSITIVITY AND SPECIFICITY: 99 percent.
LIMITATIONS: Diagnostic errors can occur due to rare sequence variations. Specific breakpoints of large deletions/duplications will not be determined; therefore, it may not be possible to distinguish mutations of similar size. This assay does not assess for non-deletional mutations within the coding or regulatory regions of the alpha globin cluster genes. Individuals carrying both a deletion and duplication within the alpha globin gene cluster may appear to have a normal number of alpha globin gene copies. Rare syndromic or acquired forms of alpha thalassemia associated with ATRX mutations will not be detected.

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	INTERPRETIVE INFORMATION: Hemoglobin Evaluation Reflexive Cascade 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.
Alpha Globin (HBA1 and HBA2) Del/Dup Rst	Performed

VERIFIED/REPORTED DATES

Procedure	Accession	Collected	Received	Verified/Reported
Hemoglobin A	22-227-104130	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Hemoglobin A2	22-227-104130	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Hemoglobin F	22-227-104130	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Hemoglobin S	22-227-104130	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Hemoglobin C	22-227-104130	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Hemoglobin E	22-227-104130	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Hemoglobin - Other	22-227-104130	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Sickle Cell Solubility	22-227-104130	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Hemoglobin, Capillary Electrophoresis	22-227-104130	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Hemoglobin Evaluation	22-227-104130	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Beta Globin Full Gene Sequencing	22-227-104130	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Beta Globin (HBB) Del/Dup Result	22-227-104130	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Alpha Thalassemia HBA1 and HBA2 Seq	22-227-104130	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Gamma Globin (HBG1 and HBG2) Sequencing	22-227-104130	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Hemoglobin Cascade Interpretation	22-227-104130	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Alpha Globin (HBA1 and HBA2) Del/Dup Rst	22-227-104130	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00

END OF CHART

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