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

Physician: Doctor, Example

Patient: Patient, Example

DOB	9/3/2023
Gender:	Female
Patient Identifiers:	01234567890ABCD, 012345
Visit Number (FIN):	01234567890ABCD
Collection Date:	00/00/0000 00:00

Alpha Globin (HBA1 and HBA2) Sequencing and Deletion/Duplication

ARUP test code 2011708

HBA Seq, Del/Dup Interp

Positive

RESULT
Two pathogenic deletions, resulting in the deletion of three of the four alpha globin gene copies, were detected in the alpha gene cluster.

DNA VARIANTS
Classification: Pathogenic
Deletion: --SEA; Heterozygous

Classification: Pathogenic
Deletion: -alpha3.7; Heterozygous

Predicted Overall Genotype: --/-a

INTERPRETATION
Two pathogenic deletions were detected by deletion/duplication analysis of the alpha globin gene cluster and its HS-40 regulatory region. The --SEA alpha globin gene deletion indicates the deletion of the HBM, HBA2, HBA1 and HBQ1 globin genes on a single chromosome. In addition, the detection of the 3.7kb deletion indicates a single functional hybrid HBA2-HBA1 alpha globin gene on the opposite chromosome. This result is consistent with Hemoglobin H disease associated with moderate anemia, severe microcytosis, hemolysis, and splenomegaly. The clinical presentation may vary due to other genetic modifiers or co-existing conditions.

Sequence analysis of HBA1 and HBA2 was not possible for this individual, as this assay is unable to sequence the HBA2-HBA1 hybrid gene resulting from the 3.7 kb deletion and the other alpha globin gene copies have been deleted. Please refer to the background information included in this report for the clinical sensitivity and limitations of this test.

Evidence for variant classifications: The pathogenic --SEA deletion (HbVar ID: 1086) is a common large deletion observed in East Asian populations (HbVar database and references therein). This deletion removes approximately 20kb including both HBA1 and HBA2 on the same chromosome, and therefore no functional mRNA is produced. Heterozygosity for this deletion is often associated with mild anemia and microcytosis, whereas homozygosity for this deletion results in Hb Bart hydrops fetalis syndrome.

The pathogenic -alpha3.7 deletion (HbVar ID: 1076) is a common large deletion observed in numerous populations, including African, Indian, Far East and Mediterranean (HbVar database and references therein). This deletion removes approximately 3.7kb of the alpha globin cluster, resulting in a single functional alpha globin gene on the affected chromosome. Heterozygosity for this deletion does not result in clinical symptoms, but may be

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

mistaken for iron deficiency. Homozygosity for this deletion is often associated with mild anemia and microcytosis.

RECOMMENDATIONS
Medical management should rely on clinical findings and family history. Carrier screening should be offered to the patient's future reproductive partner, since depending on the partner's alpha globin genotype, the couple's offspring may be a risk for Hemoglobin H disease or hydrops fetalis associated with hemoglobin Bart. Family members should be offered carrier testing for the identified deletions. Genetic consultation is recommended.

COMMENTS
Reference Sequences: GenBank # NM_000558.5 (HBA1), NM_000517.6 (HBA2), NG_000006.1 (Alpha globin gene cluster)
Nucleotide numbering begins at the "A" of the ATG initiation codon.
Likely benign and benign variants are not reported.

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

This result has been reviewed and approved by [redacted]

BACKGROUND INFORMATION: Alpha Globin (HBA1 and HBA2) Sequencing and 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 variants of a single HBA2 globin gene (-a/aa) and is clinically asymptomatic (silent carrier). Alpha (0) thalassemia (trait) is caused by variants of both HBA2 globin genes (-a/-a) or variants in the HBA1 and HBA2 globin genes on the same chromosome (--/aa) and results in mild microcytic anemia. Hemoglobin H disease occurs due to variants of three alpha globin genes (--/-a) and results in hemolysis with Heinz bodies, moderate anemia, and splenomegaly. Hb Bart Hydrops Fetalis Syndrome results when variants 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. Nondeletional alpha globin variants may be pathogenic or benign; both may result in an abnormal protein detectable by hemoglobin evaluation. Pathogenic nondeletional variants often have a more severe effect than single gene deletions.
INCIDENCE: Carrier frequency in Mediterranean (1:30-50), Middle Eastern, Southeast Asian (1:20), African, African American (1:3).
INHERITANCE: Autosomal recessive.
CAUSE: Pathogenic variants in the alpha globin gene cluster.
CLINICAL SENSITIVITY: 99 percent.
METHODOLOGY: Bidirectional sequencing of the HBA1 and HBA2 coding regions, intron-exon boundaries and 3' polyadenylation signal. Multiplex ligation-dependent probe amplification (MLPA) of the alpha globin gene cluster (HBZ, HBM, HBA1, HBA2, HBQ1) and its HS-40 regulatory region.
ANALYTICAL SENSITIVITY AND SPECIFICITY: 99 percent.
LIMITATIONS: Diagnostic errors can occur due to rare sequence variations. Sequence analysis will not detect all regulatory region variants or variants in alpha globin cluster genes other than HBA1 and HBA2. Sequencing of both HBA1 and HBA2 may not be possible in individuals harboring large alpha globin deletions on both alleles. This assay is unable to sequence HBA2-HBA1 fusion genes; thus, HBA1 or HBA2 sequence variants occurring in cis with a 3.7 kb deletion or other HBA2-HBA1 hybrid gene will not be detected (e.g. HbG Philadelphia will not be detected when in cis with the 3.7 kb deletion). It may not be possible to determine phase of identified sequence variants. Specific breakpoints of large deletions/duplications will not be determined; therefore, it may not be possible to distinguish variants of similar size. 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 variants will not be detected.

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.

HBA Seq, Del/Dup Specimen	Whole Blood

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

VERIFIED/REPORTED DATES

Procedure	Accession	Collected	Received	Verified/Reported
HBA Seq, Del/Dup Interp	23-286-123318	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
HBA Seq, Del/Dup Specimen	23-286-123318	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