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

Physician: Doctor, Example

Patient: Patient, Example

DOB	Unknown
Gender:	Unknown
Patient Identifiers:	01234567890ABCD, 012345
Visit Number (FIN):	01234567890ABCD
Collection Date:	00/00/0000 00:00

Alpha Thalassemia (HBA1 and HBA2) Deletion/Duplication with reflex to Hb Constant Spring

ARUP test code 3003651

HBA DDCS Interpretation

See Note

Indication for testing: Carrier screening or diagnostic testing for alpha thalassemia.

RESULT
One pathogenic deletion, resulting in the deletion of one alpha globin gene copy, and one copy of hemoglobin Constant Spring (HbCS) were detected in the alpha globin gene cluster.

DNA VARIANTS
Classification: Pathogenic Deletion: -alpha3.7; Heterozygous

Classification: Pathogenic Gene: HBA2
Nucleic Acid Change: c.427T>C; Heterozygous
Amino Acid Alteration: p.Ter143Gln Commonly Known As: Hb Constant Spring

Predicted Overall Genotype: -a/aCSa

INTERPRETATION
One copy of the 3.7kb deletion was identified by deletion/duplication analysis of the alpha globin gene cluster. In addition, the pathogenic HbCS variant was detected in the alpha globin gene, HBA2. The 3.7kb deletion removes the region of HBA2 harboring HbCS; therefore, the HbCS variant is predicted to be heterozygous and located on opposite chromosome from the 3.7kb deletion. This individual is predicted to have one functional alpha globin gene on each chromosome consistent with alpha thalassemia trait, which is often associated with mild anemia and microcytosis. The clinical presentation may vary due to other genetic modifiers or coexisting conditions.

Evidence for variant classifications: The pathogenic -alpha3.7 deletion 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 mistaken for iron deficiency. Homozygosity for this deletion is often associated with mild anemia and microcytosis.

The Hb Constant Spring variant (HbCS, HBA2: c.427T>C; p.Ter143Gln, also known as Ter142Gln when numbered from the mature protein, rs41464951) is usually asymptomatic in the heterozygous state, but may be associated with microcytosis and mild hypochromia. Homozygosity for HbCS is characterized by overt hemolytic anemia, jaundice and splenomegaly, while HbCS paired with an alpha zero-thalassemia deletion commonly results

in HbH disease (Lie-Injo 1974, Nguyen 2014, HbVar database). This variant is reported in ClinVar (Variation ID: 15624), and is found in the general population with an overall allele frequency of 0.006% (16/274,340 alleles) in the Genome Aggregation Database. This variant abolishes the canonical termination codon, resulting in an unstable, elongated protein (HbVar database). Based on available information, the HbCS variant is considered to be pathogenic.

RECOMMENDATIONS
Medical management should rely on clinical findings and family history. If clinical findings are not consistent with the identified variants, consider alpha globin gene sequencing. Screening for alpha globin variants should be offered to the reproductive partner of this individual. Family members should be offered testing for the identified variants. Genetic consultation is recommended.

COMMENTS
Reference Sequences: GenBank # NM_000517.4 (HBA2), NG_000006.1 (alpha globin gene cluster) Nucleotide numbering begins at the "A" of the ATG initiation codon.

REFERENCES
Link to HbVar database for the 3.7kb deletion: http://globin.bx.psu.edu/cgi-bin/hbvar/query_vars3?mode=output&display_format=page&i=1076

Link to HbVar database for Hb Constant Spring: http://globin.bx.psu.edu/cgi-bin/hbvar/query_vars3?mode=output&display_format=page&i=703

Lie-Injo L et al. Homozygous state for Hb Constant Spring (slow-moving Hb X components). Blood. 1974

INTERPRETIVE INFORMATION: Alpha Thal (HBA1/2) DelDup w/rflx HbCS

Characteristics of Alpha Thalassemia: Decreased or absent synthesis of the hemoglobin (Hb) alpha-chain resulting in clinical presentations ranging from asymptomatic silent carriers to severe anemia and fetal lethality. Alpha thalassemia silent carrier commonly results from deletion of a single alpha globin gene (-a/aa) and is clinically asymptomatic. Alpha thalassemia trait may be caused by deletion of a single alpha globin gene from both chromosomes (-a/-a), or deletion of the HBA1 and HBA2 globin genes from the same chromosome (--/aa). Heterozygosity for Hb Constant Spring (HbCS) is usually asymptomatic but may be associated with mild microcytic anemia. Homozygous HbCS is characterized by overt hemolytic anemia, jaundice and splenomegaly. Hemoglobin H disease occurs due to inactivation of three alpha globin genes and results in hemolysis with Heinz bodies, moderate anemia, and splenomegaly. Hb Bart hydrops fetalis syndrome results from deletion of all four alpha globin genes (--/--) and is lethal in the fetal or early neonatal period. Alpha globin gene duplication results in three or more active alpha globin genes on a single chromosome.
Epidemiology: Carrier frequency of alpha thalassemia in African, African-American (1:3), Mediterranean (1:30-50), Middle Eastern, Southeast Asian (1:20).
Inheritance: Autosomal recessive.
Cause: Pathogenic variants in the alpha globin gene cluster (HBZ, HBM, HBA2, HBA1, HBQ1) or regulatory region.
Clinical Sensitivity: Varies by ethnicity, at least 90 percent.
Methodology: Multiplex ligation-dependent probe amplification (MLPA) for the HBZ, HBM, HBA2, HBA1, and HBQ1 genes, the HS-40 regulatory region, and Hb Constant Spring (HbCS) HBA2 c.427T>C; p.Ter143Gln. To determine copy number of HbCS in absence of a concurrent deletion of HBA2, PCR and bidirectional sequencing for HbCS is performed.
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 variants of similar size. Non-deletional variants within the coding or regulatory regions of the alpha globin cluster genes, other than HbCS, will not be targeted. 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 gene 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 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.

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

VERIFIED/REPORTED DATES

Procedure	Accession	Collected	Received	Verified/Reported
HBA DDCS Interpretation	21-144-118615	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