HOTLINE: Effective January 11, 2021

New Test **3003656** **Alpha Thalassemia (*HBA1* and *HBA2*) Deletion/Duplication with reflex to Hb Constant Spring, Fetal** **HBA DDCSFE**

Available Now

Additional Technical Information

Patient History Form for Fetal Molecular Testing

Methodology: Multiplex Ligation-dependent Probe Amplification with Sanger sequencing confirmation of HbCS
Performed: Varies
Reported: 10 days

Specimen Required: Collect: **Fetal Specimen**: Two T-25 flasks at 80 percent confluency of cultured amniocytes or CVS. **If the client is unable to culture amniocytes, this can be arranged by contacting ARUP Client Services at (800) 522-2787.** Or amniotic fluid.
AND Maternal Specimen: Lavender (EDTA), Pink (K_2EDTA), or Yellow (ACD Solution A or B).
Specimen Preparation: **Cultured Amniocytes or Cultured CVS**: Fill flasks with culture media. Transport two T-25 flasks at 80 percent confluency of cultured amniocytes or cultured CVS. Backup cultures must be retained at the client's institution until testing is complete.
OR Amniotic Fluid: Transport 10 mL unspun fluid.
AND Maternal Specimen: Transport 2 mL whole blood. (Min: 1 mL)
Storage/Transport Temperature: **Amniotic Fluid:** Room temperature.
Cultured Fetal Cells: CRITICAL ROOM TEMPERATURE. Must be received within 48 hours of shipment due to viability of cells.
Maternal Specimen: Room temperature.
Remarks: **Please contact an ARUP genetic counselor at 800-242-2787 x2141 prior to sample submission.** Patient History Form is available on the ARUP Web site or by contacting ARUP Client Services at (800) 522-2787.
Unacceptable Conditions:
Stability (collection to initiation of testing): **Fetal Specimen:** Room temperature: 48 hours; Refrigerated: Unacceptable; Frozen: Unacceptable
Maternal Specimen: Room temperature: 7 days; Refrigerated: 1 month

Reference Interval: By report

Interpretive Data:
Background Information: Alpha Globin (*HBA1* and *HBA2*) Deletion/Duplication
Characteristics: 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 detected. Fetuses 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.

Note: If a concurrent deletion of *HBA2* is not identified, PCR and bidirectional sequencing for the HbCS copy number will be performed. Additional charges apply.

CPT Code(s): 81269; 81265; if reflexed, add 81257

New York DOH Approved.

HOTLINE NOTE: Refer to the Test Mix Addendum for interface build information.