TEST CHANGE

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

3003656, HBA DDCSFE

Specimen Requirements:	
Patient Preparation:	
Collect:	Fetal Specimen: Cultured amniocytes OR cultured chorionic villus sampling (~~or Cultured~~ CVS). ~~AND~~ Maternal Specimen: Refer to Maternal Cell Contamination, Maternal Specimen (0050608) for maternal specimen requirements.~~Whole Blood Specimen: Lavender (EDTA), pink (K2EDTA), or yellow (ACD solution A or B).~~
Specimen Preparation:	Transport: Two T-25 flasks of 80% confluent~~Cultured Amniocytes or Cultured CVS: Transfer~~ cultured amniocytes OR Two T-25 flasks of 80% confluent cultured chorionic villus sampling (CVS). Cultured amniocytes or ~~or~~ cultured CVS is required for testing. If submitting uncultured (direct) amniotic fluid or (direct) CVS and testing is desired on a cultured specimen, add Cell Culture for Genetic Testing (3020627). If transporting flasks, the client is responsible for maintaining backup~~to two T-25 flasks at 80 percent confluence. (Min: one T-25 flask at 80 percent confluence). Backup~~ cultures ~~must be retained~~ at the client~~'s~~ institution~~. until testing is complete.~~ If ARUP receives cultured fetal cells~~a sample~~ below ~~the~~ minimum confluence, Cell Culture for Genetic Testing (3020627~~Cytogenetics Grow and Send (ARUP test code 0040182~~) will be added ~~on~~ by ARUP~~, and additional charges will apply. If clients are unable to culture specimens, Cytogenetics Grow and Send should be added to initial order. Maternal Whole Blood Specimen: Transport 2 mL whole blood. (Min: 1 mL)~~
Transport Temperature:	Preferred transport: Room temperature. Preferred shipment: Within two days of collection or confluence.~~Cultured Amniocytes or CVS : CRITICAL ROOM TEMPERATURE. Must be received within 48 hours of collection due to viability of cells.~~ ~~Maternal Whole Blood Specimen: Room temperature.~~
Unacceptable Conditions:	Frozen specimens.
Remarks:	Counseling and informed consent are recommended for genetic testing. Consent forms are linked above. New York Clients: Informed consent is required with submission.
Stability:	~~Cultured Amniocytes or Cultured CVS:~~ Room temperature: 2 days~~48 hours~~; Refrigerated: Unacceptable; Frozen:

	Unacceptable ~~Maternal Whole Blood Specimen : Room temperature: 7 days; Refrigerated: 1 month; Frozen: Unacceptable~~
Methodology:	Multiplex Ligation-Dependent Probe Amplification (MLPA) / Sequencing
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 Codes:	81269; 81265; Fetal Cell Contamination (FCC); if reflexed, add 81257
New York DOH Approval Status:	This test is New York DOH approved.

Interpretive Data:

Refer to report.~~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. Nondeletional 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.~~

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

Reference Interval:

Refer to report