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

Physician: Doctor, Example

Patient: Patient, Example

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

Hemophilia A (F8) 2 Inversions with Reflex to Sequencing and Reflex to Deletion/Duplication

ARUP test code 3004232

F8 COMP Specimen	Whole Blood
Family History for Hemophilia A (F8)	Unknown
Symptoms for Hemophilia A (F8)	Unknown
Hemophilia A (F8) Interpretation	Positive

Inversion Analysis: Negative for pathogenic variants, therefore, F8 sequencing was performed.
Sequencing: Positive for a pathogenic variant, therefore, F8 deletion/duplication testing was not performed.

RESULT
One pathogenic variant was detected in the F8 gene.

PATHOGENIC VARIANT
Gene: F8 (NM_000132.3)
Nucleic Acid Change: c.6089G>A; Hemizygous
Amino Acid Alteration: p.Ser2030Asn
Inheritance: X-linked

INTERPRETATION
One pathogenic variant, c.6089G>A; p.Ser2030Asn, was detected in the F8 gene by massively parallel sequencing. This molecular result is consistent with a diagnosis of hemophilia A. All of this individual's female offspring, but none of the male offspring, will inherit the pathogenic variant.

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

Evidence for variant classification:
The F8 c.6089G>A; p.Ser2030Asn variant (rs369414658), also known as Ser2011Asn, is reported in the literature in multiple individuals affected with mild hemophilia A (see F8 database and references therein, Lannoy 2015, Liu 1998, Markoff 2009, Repesse 2007) and is considered a pathogenic founder variant in the Belgian population (Lannoy 2015). This variant is reported in ClinVar (Variation ID: 439683). It is found in the non-Finnish European population with an allele frequency of 0.006% (5/81740 alleles, including 3 hemizygotes) in the Genome Aggregation Database. The serine at codon 2030 is highly conserved, but computational analyses are uncertain whether this variant is

neutral or deleterious (REVEL: 0.573). Based on available information, this variant is considered to be pathogenic.

RECOMMENDATIONS
This individual should be followed at a hemophilia treatment center. Genetic consultation is indicated, including a discussion of medical screening and management. At-risk family members should be offered testing for the identified pathogenic variant (Familial Targeted Sequencing, ARUP test code 3005867).

COMMENTS
Unless otherwise specified, confirmation by Sanger sequencing was not performed for variants with acceptable quality metrics. 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:
NONE

REFERENCES
Factor VIII Variant Database: https://f8-db.eahad.org/

Lannoy N et al. Overrepresentation of missense mutations in mild hemophilia A patients from Belgium: founder effect or independent occurrence? Thromb Res. 2015 135:1057-1063. PMID: 25824987.

Liu M et al. A domain mutations in 65 haemophilia A families and molecular modelling of dysfunctional factor VIII proteins. Br J Haematol. 1998 103:1051-1060. PMID: 9886318.

Markoff A et al. Combined homology modelling and evolutionary significance evaluation of missense mutations in blood clotting factor VIII to highlight aspects of structure and function. Haemophilia. 2009 15:932-941. PMID: 19473423.

Repesse Y et al. Factor VIII (FVIII) gene mutations in 120 patients with hemophilia A: detection of 26 novel mutations and correlation with FVIII inhibitor development. J Thromb Haemost. 2007 5:1469-1476. PMID: 17445092.

This result has been reviewed and approved by [redacted]

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

BACKGROUND INFORMATION: Hemophilia A (F8) 2 Inversions with Reflex to Sequencing and Reflex to Deletion/Duplication
CHARACTERISTICS: Hemophilia A is characterized by deficiency of factor VIII clotting activity. Less than 1 percent factor VIII activity results in severe deficiency associated with spontaneous joint or deep muscle bleeding. Moderate deficiency (1-5 percent activity) and mild deficiency (6-40 percent activity) are associated with prolonged bleeding after tooth extractions, surgery, or injuries, and recurrent or delayed wound healing. Female carriers of hemophilia A may have increased bleeding tendencies.
EPIDEMIOLOGY: 1 in 5,000 live male births worldwide
CAUSE: Pathogenic F8 germline variants
INHERITANCE: X-linked recessive. In the estimated 30 percent of cases that appear to be de novo, the mother is found to be a carrier at least 80 percent of the time.
PENETRANCE: 100 percent in males. Approximately 30 percent of female carriers have factor VIII activity levels of less than 40 percent and are at risk for bleeding symptoms typically consistent with mild hemophilia A.
CLINICAL SENSITIVITY: 98 percent
GENE TESTED: F8 (NM_000132.4)
METHODOLOGY: F8 intron 22-A and intron 1 inversions detected by inverse PCR and electrophoresis. Capture of all coding exons and exon-intron junctions of the F8 gene, followed by massively parallel sequencing. Sanger sequencing performed as necessary to fill in regions of low coverage and confirm reported variants. Multiplex ligation-dependent probe amplification (MLPA) of the F8 gene.
ANALYTICAL SENSITIVITY/SPECIFICITY: The analytical sensitivity and specificity for inversion analysis and MLPA is 99 percent. The analytical sensitivity of sequencing is approximately 99 percent for single nucleotide variants (SNVs) and greater than 93 percent for insertions/duplications/deletions from 1-10 base pairs in size. Variants 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 or carrier status for hemophilia A. This test only detects variants within the coding regions and intron-exon boundaries of the F8 gene. Variants in regions that are not included in the preferred transcript are not detected. Regulatory region variants and deep intronic variants, other than the type 1 or type 2 intron 22-A and intron 1 inversions, will not be identified. Rare F8 intron 22-A and intron 1 inversions with different breakpoints may not be detected by this assay. Breakpoints for large deletions/duplications will not be determined. Single exon deletion/duplications may not be detected based on the breakpoints of the rearrangement. Deletions/duplications/insertions of any size may not be detected by massively parallel sequencing. Diagnostic errors can occur due to rare sequence variations. In some cases, variants may not be identified due to technical limitations in the presence of pseudogenes, repetitive, or homologous regions. This assay may not detect low-level mosaic or somatic variants associated with disease. 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 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.

VERIFIED/REPORTED DATES

Procedure	Accession	Collected	Received	Verified/Reported
F8 COMP Specimen	22-307-111087	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Family History for Hemophilia A (F8)	22-307-111087	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Symptoms for Hemophilia A (F8)	22-307-111087	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Hemophilia A (F8) Interpretation	22-307-111087	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