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

Physician: Doctor, Example

Patient: Patient, Example

DOB	9/5/2020
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)	No
Symptoms for Hemophilia A (F8)	Yes
Hemophilia A (F8) Interpretation	Positive Inversion Analysis: Negative for pathogenic variants, therefore, F8 sequencing was performed. Sequencing: Negative for pathogenic variants, therefore, F8 deletion/duplication testing was performed. Deletion/Duplication Analysis: Positive for a pathogenic variant. INDICATION FOR TESTING Confirm diagnosis RESULT One pathogenic deletion was detected in the F8 gene. PATHOGENIC VARIANT Gene: F8 (NM_000132.3) Nucleic Acid Change: Deletion of exon 1; Hemizygous Inheritance: X-linked INTERPRETATION One pathogenic variant, a deletion of exon 1, was identified in the F8 gene by Multiplex Ligation-dependent Probe Amplification (MLPA) analysis. This molecular result is consistent with a diagnosis of hemophilia A. All of this individual's future female offspring, and none of the future male offspring, will inherit the variant. Since this deletion includes the first exon of the F8 gene, and the breakpoints of the deletion cannot be determined by this assay, the deletion may extend upstream of the F8 gene. No additional pathogenic variants were detected by inversion testing or massively parallel sequencing. Please refer to the background information included in this report for the clinical sensitivity and limitations of this test. Evidence for variant classification: The F8 exon 1 deletion is reported in the literature in multiple individuals affected with severe hemophilia A (see link to Factor VIII gene database and

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

references therein, Green 2008, Johnsen 2017, Rossetti 2007), and a similar variant is reported in ClinVar (Variation ID: 10095). 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 variant (Deletion/Duplication Analysis by MLPA, ARUP test code 3003144).

COMMENTS
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; reportable variants are confirmed by Sanger sequencing:
NONE

REFERENCES
Link to Factor VIII gene database:
http://f8-db.eahad.org/index.php

Green PM et al. Haemophilia A mutations in the UK: results of screening one-third of the population. Br J Haematol. 2008 Oct;143(1):115-28.

Johnsen JM et al. Novel approach to genetic analysis and results in 3000 hemophilia patients enrolled in the My Life, Our Future initiative. Blood Adv. 2017 May 18;1(13):824-834.

Rossetti LC et al. Sixteen novel hemophilia A causative mutations in the first Argentinian series of severe molecular defects. Haematologica. 2007 Jun;92(6):842-5.

This result has been reviewed and approved by [redacted]

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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.

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VERIFIED/REPORTED DATES

Procedure	Accession	Collected	Received	Verified/Reported
F8 COMP Specimen	22-099-400836	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Family History for Hemophilia A (F8)	22-099-400836	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Symptoms for Hemophilia A (F8)	22-099-400836	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Hemophilia A (F8) Interpretation	22-099-400836	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00

END OF CHART

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