ARUP LABORATORIES
800-522-2787 | aruplab.com
500 Chipeta Way, Salt Lake City, UT 84108-1221
Jonathan R. Genzen, MD, PhD, Chief Medical Officer

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

Hereditary Retinoblastoma (RB1) Sequencing and Deletion/Duplication

ARUP test code 3005696

RB1 Specimen	Whole Blood
RB1 Interp	Positive

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

RESULT
One pathogenic variant was detected in the RB1 gene.

PATHOGENIC VARIANT
Gene: RB1 (NM_000321.2)
Nucleic Acid Change: c.1891C>T; Heterozygous
Amino Acid Alteration: p.Gln631Ter
Inheritance: Autosomal dominant

INTERPRETATION
One pathogenic variant, c.1891C>T; p.Gln631Ter, was detected in the RB1 gene by massively parallel sequencing. Pathogenic germline variants in RB1 are associated with autosomal dominant retinoblastoma (MIM: 180200). This individuals future offspring have a 50 percent chance of inheriting 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 RB1 c.1891C>T; p.Gln631Ter variant (rs1217977493) is reported in the literature in an individual with retinoblastoma (Kiran, 2003). The variant is also reported in the ClinVar database (Variation ID: 860313). It is absent from the Genome Aggregation Database, indicating it is not a common polymorphism. This variant induces an early termination codon and is predicted to result in a truncated protein or mRNA subject to nonsense-mediated decay. Additionally, several downstream truncating variants have been described in individuals with retinoblastoma and are considered to be pathogenic (Taylor, 2007). Based on available information, this variant is classified as pathogenic.

RECOMMENDATIONS
Genetic consultation is indicated, including a discussion of medical screening and management. At-risk family members should be offered testing for the identified pathogenic RB1 variant (Familial Targeted Sequencing, ARUP test code 3005867).

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:
NONE

REFERENCES
Kiran VS, et al. Mutational screening of the RB1 gene in Indian patients with retinoblastoma reveals eight novel and several recurrent mutations. Hum Mutat. 2003;22(4):339. PMID: 12955724.

Taylor M, et al. Genotype-phenotype correlations in hereditary familial retinoblastoma. Hum Mutat. 2007;28(3):284-293. PMID: 17096365.

BACKGROUND INFORMATION: Hereditary Retinoblastoma (RB1)
Sequencing and Deletion/Duplication
CHARACTERISTICS: Retinoblastoma is a malignant tumor of the retina that typically occurs in children under 5 years of age. Hereditary retinoblastoma, caused by a single germline pathogenic variant in the RB1 gene, predisposes individuals to retinoblastoma and other nonocular tumors (including pinealoblastoma, osteosarcoma, soft tissue sarcoma, and melanoma).

EPIDEMIOLOGY: Retinoblastoma affects approximately 1:15,000 to 1:20,000 live births. Hereditary retinoblastoma accounts for approximately 10 percent of retinoblastoma cases.

CAUSE: Pathogenic germline variants in the RB1 gene

INHERITANCE: Autosomal dominant

PENETRANCE: Complete penetrance, except for fewer than 10 percent of families that show a low-penetrance phenotype with reduced expressivity

CLINICAL SENSITIVITY: Varies, dependent on phenotype

GENE TESTED: RB1* (NM_000321)

*One or more exons are not covered by sequencing and/or deletion/duplication analysis; see limitations section below.

METHODOLOGY: Probe hybridization-based capture of all coding exons and exon-intron junctions of the RB1 gene, followed by massively parallel sequencing. Sanger sequencing was performed as necessary to fill in regions of low coverage and to confirm reported variants that do not meet acceptable quality metrics. A proprietary bioinformatic algorithm was used to detect large (single exon-level or larger) deletions or duplications in the indicated genes. Large deletions/duplications confirmed using an orthogonal exon-level microarray. Human genome build 19 (Hg 19) was used for data analysis.

ANALYTICAL SENSITIVITY/SPECIFICITY: The analytical sensitivity is approximately 99 percent for single nucleotide variants (SNVs) and greater than 93 percent for insertions/duplications/deletions (indels) from 1-10 base pairs in size. Indels greater than 10 base pairs may be detected, but the analytical sensitivity may be reduced. Deletions of two exons or larger are detected with sensitivity greater than 97 percent; single exon deletions are detected with 62 percent sensitivity. Duplications of three exons or larger are detected at greater than 83 percent sensitivity. Specificity is greater than 99.9 percent for all variant classes.

LIMITATIONS: A negative result does not exclude a heritable form of retinoblastoma. This test only detects variants within the coding regions and intron-exon boundaries of the RB1 gene. Deletions/duplications/insertions of any size may not be detected by massively parallel sequencing. Regulatory region variants and deep intronic variants will not be identified. Precise breakpoints for large deletions or duplications are not determined in this assay and single exon deletions/duplications may not be detected based on the breakpoints of the rearrangement. The actual breakpoints for the deletion or duplication may extend beyond or be within the exon(s) reported. This test is not intended to detect duplications of two or fewer exons in size, though these may be identified. Single exon deletions are reported but called at a lower sensitivity. Diagnostic errors can occur due to rare sequence variations. In some cases, variants may not be identified due to technical limitations caused by the presence of pseudogenes, repetitive, or homologous regions. This test is not intended to detect low-level mosaic or somatic variants, gene conversion events, complex inversions, translocations, mitochondrial DNA (mtDNA) variants, or repeat expansions. Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation. Noncoding transcripts were not analyzed.

The following exons may have reduced sequencing sensitivity due to technical limitations of the assay:
RB1 (NM_000321) 22

Deletions/duplications will not be called for the following exons:
RB1 (NM_000321) 22

This test was developed and its performance characteristics determined by ARUP Laboratories. It has not been cleared or approved by the U.S. 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
RB1 Specimen	22-319-100757	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
RB1 Interp	22-319-100757	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