Retinoblastoma: *RB1* Gene Sequencing

**Test Code:** SRB1X  
**Turnaround time:** 6 weeks  
**CPT Codes:** 81479 x1

### Condition Description

Retinoblastoma (RB) is rare malignant tumor of the retina that occurs primarily in infancy and childhood. Approximately 60% of affected individuals have unilateral RB (affecting one eye) while the remaining 40% have bilateral RB (affecting both eyes). RB typically presents in the first five years of life; unilateral RB typically occurs at an average age of 24 months and bilateral RB typically occurs at an average age of 15 months. Retinoblastoma occurs in both hereditary and non-hereditary forms. Virtually all bilateral RB and multifocal RB as well as 15% of unilateral RB is hereditary. It is estimated that about 55% of all retinoblastoma is hereditary.

Hereditary RB is inherited in an autosomal dominant manner. Hereditary RB is caused by mutations in the tumor-suppressor gene *RB1* located at 13q14.2. Individuals with hereditary RB are said to have a germline mutation in *RB1*. In the majority of hereditary cases, this occurs as a *de novo* event, however in about 20% of hereditary cases, affected individuals inherit a mutation in *RB1* from a parent. In 90-95% of these patients an *RB1* mutation can be detected in their blood. Individuals with a *RB1* mutation have a predisposition to developing RB and other cancers, such as osteosarcomas and pinealoma.

For patients with suspected RB, sequence analysis is recommended as the first step in mutation identification. For patients in whom mutations are not identified by full gene sequencing, deletion/duplication analysis is appropriate.

### References:

- GeneReview
- Aerts I et al. Retinoblastoma. Orphanet J Rare Diseases. 2006;1:31
- OMIM #614041: RB1 gene
- OMIM #180200: RB

### Genes

*RB1*

### Indications

This test is indicated for:

- Confirmation of a clinical diagnosis of retinoblastoma.

### Methodology

**Next Generation Sequencing:** In-solution hybridization of all coding exons is performed on the patient's genomic DNA. Although some deep intronic regions may also be analyzed, this assay is not meant to interrogate most promoter regions, deep intronic regions, or other regulatory elements, and does not detect single or multi-exon deletions or duplications. Direct sequencing of the captured regions is performed using next generation sequencing. The patient's gene sequences are then compared to a standard reference sequence. Potentially causative variants and areas of low coverage are Sanger-sequenced. Sequence variations are classified as pathogenic, likely pathogenic, benign, likely benign, or variants of unknown significance. Variants of unknown significance may require further studies of the patient and/or family members.

### Detection

**Clinical Sensitivity:** 70% of mutations can be identified by sequencing analysis. Mutations in the promoter region, some mutations in the introns and other regulatory element mutations cannot be detected by this analysis. Large deletions will not be detected by this analysis. Results of molecular analysis should be interpreted in the context of the patient's clinical and/or biochemical phenotype.

**Analytical Sensitivity:** ~99%

### Specimen Requirements

**Submit only 1 of the following specimen types**

**Type:** Whole Blood (EDTA)

**Specimen Requirements:**
- EDTA (Purple Top)
- Infants and Young Children (2 years of age to 10 years old): 3-5 ml
- Older Children & Adults: 5-10 ml
- Autopsy: 2-3 ml unclotted cord or cardiac blood

**Specimen Collection and Shipping:**
Ship sample at room temperature for receipt at EGL within 24 hours of collection. Do not refrigerate or freeze.

**Type: DNA, Isolated**

**Specimen Requirements:**
Microtainer
8µg
Isolation using the Perkin Elmer™ Chemagen™ Automated Extraction method or Qiagen™ Puregene kit for DNA extraction is recommended.

**Specimen Collection and Shipping:**
Refrigerate until time of shipment in 100 ng/µL in TE buffer. Ship sample at room temperature with overnight delivery.

**Type: Saliva**

**Specimen Requirements:**
Oragene™ Saliva Collection Kit
Orangene™ Saliva Collection Kit used according to manufacturer instructions. Please contact EGL for a Saliva Collection Kit for patients that cannot provide a blood sample.

**Specimen Collection and Shipping:**
Please do not refrigerate or freeze saliva sample. Please store and ship at room temperature.

**Related Tests**

- Deletion/duplication analysis of the \( \text{RB1} \) gene by CGH array is available for those individuals in whom sequence analysis is negative.
- Custom diagnostic mutation analysis (KM) is available to family members if mutations are identified by targeted mutation testing or sequencing analysis.
- Prenatal testing is available only for known familial mutations to individuals who are confirmed carriers of mutations. Please contact the laboratory genetic counselor to discuss appropriate testing prior to collecting a prenatal specimen.