



impact genetics

Retinoblastoma

Genetic Test

World leaders in retinoblastoma genetics | Highest reported pick-up rate

Test Description

Genes tested

RB1, MYCN

Copy number changes

Our lab uses Multiplex Ligation-dependent Probe Amplification (MLPA) to look for whole-exon and multi-exon deletions and duplications in the *RB1* gene. Our lab also performs a QM-PCR test to measure *MYCN* copy number in any tumor sample that does not have an *RB1* mutation. This test detects a new sub-set of retinoblastoma with early age at diagnosis, distinct histology, no *RB1* mutations, and huge amplification of the *MYCN* oncogene.

[Rushlow, D. et al. 2013. *Lancet Oncology*.]

Sequence analysis

Our lab sequences the *RB1* gene core promoter and exons 1 through 27, as well as nearby flanking intronic regions. Our sequence analysis is able to detect mosaic mutations at a level of 15% or greater. We consider reported polymorphisms when designing our sequencing assays to ensure the accuracy of our sequence results.

We sequence a minimum of 25 nucleotides flanking each exon of *RB1* to detect changes in splice sites. We use *in silico* analysis and scoring to determine whether a particular change is likely to cause missplicing. In the case of an intronic variant of uncertain significance we perform RNA transcript analysis on a fresh blood sample at no added charge.

In addition, when appropriate we will test known affected and unaffected relatives at no charge to clarify variant classification.

Allele-specific PCR (AS-PCR) for eleven recurrent *RB1* mutations

Our lab uses multiplex AS-PCR screens for rapid detection of eleven recurrent *RB1* mutations, which are then confirmed by sequence analysis. The highly sensitive AS-PCR can detect the eleven mutations at mosaic levels as low as 1% mutant DNA.

Testing for methylation of the *RB1* promoter

Aberrant methylation of the *RB1* promoter leads to reduced transcription of *RB1*, and can initiate unilateral sporadic retinoblastoma in the absence of an *RB1* mutation. Our lab identifies *RB1* promoter methylation leading to retinoblastoma in approximately 12% of unilateral sporadic tumors.

RNA Analysis

In the event that no *RB1* mutation is found for bilateral or unilateral patients with a strong family history we will pursue transcript analysis to look for deep intronic changes that may impact splicing at no additional charge.

Notes

- Impact has chosen not to work with paraffin-embedded tumors for *RB1* mutation discovery. The fragmented, degraded DNA isolated from paraffin-embedded material amplifies poorly and unreliably, interfering with complete analysis.
- Where fresh or frozen tumor is not available, our lab performs a complete *RB1* mutation screen of blood. Because of our 97% sensitivity, a negative *RB1* mutation screen of blood reduces the risk of a germline mutation in a unilaterally affected sporadic patient from approximately 15% to less than 0.4%.

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Test Sensitivity | Higher sensitivity, lower total costs

- ‘Heritability’ is now recognized as a key factor in AJCC guidelines for retinoblastoma cancer.

- The new guidelines state that if certain hereditary features are present in the individual – such as a family history that suggests a hereditary trait for retinoblastoma - they will be assigned as H1 or hereditary status without molecular testing. However if these heritable traits are not present, molecular testing is mandatory before an H1 status can be assigned to an individual. In this case, the guidelines recommend the laboratory providing the molecular testing must demonstrate an assay clinical sensitivity of greater than 97%.

- Highest test sensitivity is required for retinoblastoma TNMH staging

- Impact Genetics is the only lab with high enough clinical sensitivity (97%) to assign TNMH’s ‘H0’ in unilateral patients, ruling out a heritable trait with more than 99% confidence.

Bilateral clinical sensitivity: 97%

Unilateral clinical sensitivity: 19%

19% (based on Impact Genetics’ data) of patients with unilateral retinoblastoma carry a germline RB1 mutation putting them at lifelong risk for other cancers. Previous literature has estimated that 15% of unilateral retinoblastoma patients carry the hereditary form [Knudson, A. 1971. Proc Natl Acad Sci USA.]; however, improved genetic testing has shown that many more patients carry the hereditary form. Correctly diagnosing hereditary retinoblastoma changes care and outcomes.

Unilateral retinoblastoma – are you missing cases of hereditary retinoblastoma?

	Clinical sensitivity	Undetected mutations	% germline	Residual germline risk
Lab “X”	90%	10%	x 19%	= 1.9%
Impact Genetics	97%	3%	x 19%	= 0.6%

- Important definitions

- **Clinical sensitivity:** % of individuals with retinoblastoma who are identified by the assay as positive. It is important to determine if the lab quotes their own clinical sensitivity or generic analytical sensitivity which is not relevant here.
- **Residual germline risk:** risk of a false negative hereditary genetic result. Test sensitivity directly affects the residual germline risk.

Chance a mutation will not be detected x Chance patient is a germline carrier = Residual germline risk

- Impact’s diagnostic sensitivity is updated and published in every patient report.

- As of July, 2017, 2580 clinical families have been tested.

	Mutations found	Samples tested	Clinical sensitivity
Bilateral proband	861	888	97%
Unilateral proband	144	772	19%

- If no causative mutation is found, we bank samples and re-test in the future when new science or test methods are available. There is no charge for re-issued reports.
- For family member samples, we run relative and proband samples together to verify detection of the family mutation.
- For **prenatal** cases, we include microsatellite analysis to ensure that the sample is not contaminated with maternal cells.



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Choose Impact | Excellence and expertise

- **MYCN:** Impact includes *MYCN* copy-number testing in retinoblastoma genetic testing.
- **Reports:** We strive to provide as much useful information as possible. World-renowned medical experts contribute to cases involving complex interpretation.
- **Sensitivity:** Higher sensitivity in detecting mutations reduces unnecessary clinical screening for non-carriers in the family, and enables targeted clinical screening for those at high risk, leading to more precise treatment. Our sensitivity is the highest reported from any lab in North America.
- **Re-tests:** If no mutation is found, we bank any remaining DNA and RNA and re-test in the future when new science or test methods are available. For any new findings, we re-issue our report at no added charge.
- **Experience:** We have completed analysis for more than 1800 retinoblastoma families, building a knowledge base that allows us to provide more accurate interpretation.
- **Ascribing value to variants:** We analyze all variants of uncertain significance using current *in silico* methods and take into account the knowledge we have gained from our previous test experience. In addition, we search *RB1* mutation databases for previous reports of the variant in question. Where needed to define a causative mutation, we request family member samples to test at no added charge.
- **Clinically appropriate turn-around time:**
 - Proband turn-around time is 3-6 weeks.
 - Known familial mutation turn-around time is 2-3 weeks.
 - Prenatal turn-around time is 7 business days.
- **Certified lab:** Our lab is fully accredited and certified:
 - College of American Pathologists (CAP) and CLIA '88
 - Institute for Quality Management in Healthcare (IQMH) ISO 15189
 - New York State CLEP
 - European Molecular Genetics Quality Network
- **Service excellence:** Impact is committed to exceptional customer service. Our team happily provides genetic counseling and test order support so you can spend more time with your patients.
- **Logistics:** Impact provides genetic testing services to over 25 countries and samples are routinely shipped from across the world without disruption. We provide the necessary paperwork and recommend a courier service that will reliably deliver samples.

Test Turn-Around Times

Proband.....	3-6 weeks
Known familial mutation	2-3 weeks
Prenatal	7 business days



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Our Research | *On the leading edge of retinoblastoma genetics*

In addition to using the latest research to constantly improve our test methods, we work with our founder Dr. Brenda Gallie and other RB experts to perform and publish our own research to ensure we are at the leading edge of retinoblastoma genetics. For example, our lab recently discovered a unique subset of unilateral sporadic retinoblastoma with no *RB1* tumor mutations but very high amplification of the *MYCN* oncogene. We share our mutation information in registered mutation databases for use by other researchers and clinicians. Our referring specialists have access to anonymized data from our banked patient samples including clinical information. We are open to modifying requisitions as needed to collect new and important clinical phenotype information. Our CAP compliant ethics and privacy policies ensure that appropriate consent is in place.

Examples of papers our team has contributed to:

- Mallipatna, A. et al. 2017. **Retinoblastoma Chapter 68.** *AJCC Cancer Staging Manual, Eighth Edition.* 819-830.
- Soliman, S. **Genetics and Molecular Diagnosis in Retinoblastoma - An Update.** *Asia-Pacific Journal of Ophthalmology.* 6(2): 197-207.
- Soliman, S. **Prenatal versus Postnatal Screening for Familial Retinoblastoma.** *American Academy of Ophthalmology.* 123(12): 2610-2617.
- Rushlow, D. et al. 2013. **Characterization of retinoblastomas without *RB1* mutations: genomic, gene expression, and clinical studies.** *Lancet Oncol.* 14(4): 327-334.
Discovery and characterization of a new subset of retinoblastoma with early age at diagnosis, distinct histology, no *RB1* mutations, and huge amplification of the *MYCN* oncogene.
- Rushlow, D. et al. 2009. **Detection of mosaic *RB1* mutations in families with retinoblastoma.** *Hum Mutat.* 30(5): 842-51.
We discuss the incidence of *RB1* mutational mosaicism and the use of highly sensitive methods to detect low level mosaics.
- Prigoda-Lee, N. et al. 2009. **Retinoblastoma: the evidence does matter.** *Cell Oncol.* 31(3): 249-50.
- Zhang, E. et al. 2008. **Patterns of missplicing caused by *RB1* gene mutations in patients with retinoblastoma and association with phenotypic expression.** *Hum Mutat.* 29(4): 475-84.
This study analyzes the effect of various *RB1* mutations on the *RB1* mRNA transcript. It also concludes that pathogenic mutations deep in the *RB1* introns are likely to be rare.
- Richter, S. et al. 2003. **Sensitive and efficient detection of *RB1* gene mutations enhances care for families with retinoblastoma.** *Am J Hum Genet.* 72(2): 253-69.
We show that highly sensitive and efficient molecular methods to identify a family's *RB1* mutation reduce healthcare costs, and avoid unnecessary examinations under anesthetic for relatives who do not carry the mutation.

Please visit impactgenetics.com for more information and updates on our research.