

GENETIC DIAGNOSTIC LABORATORY
UNIVERSITY OF PENNSYLVANIA SCHOOL OF MEDICINE
DEPARTMENT OF GENETICS
Tel: (215) 573-9161 • Fax: (215) 573-5940
CLIA ID: 39D0893887

Retinoblastoma (RB1)

Background: Retinoblastoma (RB) is a malignant tumor of the developing retina that occurs in children, usually before the age of five years. Retinoblastoma may be unilateral or bilateral. Approximately 60% of patients have unilateral RB with a mean age at diagnosis of 24 months; approximately 40% of patients have bilateral RB with a mean age at diagnosis of 15 months. Germline mutations in the *RB1* gene (chromosomal location 13q14) predispose individuals to the ocular tumor, and they are then at increased risk of developing other RB-related (non-ocular) tumors.

Assay: Full sequencing of *RB1* gene in DNA isolated from blood or tumor
Deletion/Duplication analysis of *RB1* gene in DNA isolated from blood or tumor

Utility: Diagnostic confirmation and prenatal diagnosis

Sensitivity: The probability that a *RB1* gene mutation will be detected in an index case depends upon whether the tumor is unilateral or bilateral, unifocal or multi-focal, whether we test tumor or blood, and whether the family history is positive or negative for disease. If the tumor is/was bilateral the sensitivity is 89% for a sequencing mutation to be present. If the tumor is/was unilateral and there is no family history, tumor tissue should be tested first. If tumor is not available, the sensitivity for a germline mutation being present is ~10%. Sensitivity of testing if tumor is provided is ~77%. Mutations in non-coding sequences, deletions, or duplications will not be detected by sequencing but may be detected by the deletion/duplication assay.

Turn around: Full sequencing of *RB1* gene in DNA isolated from blood or flash frozen tumor: 8-12 weeks
Full sequencing of *RB1* gene in DNA isolated from tumor in paraffin block: 12-15 weeks
Deletion/Duplication assay in blood or tumor: 4-6 weeks
Familial mutation analysis of *RB1* gene in DNA isolated from blood: 2-3 weeks
Prenatal diagnosis of *RB1* sequencing or dup/del mutation in DNA isolated from CVS or amniocytes: 1-3 weeks

Fees: \$1720 full gene sequencing of DNA isolated from blood or flash frozen tumor, with automatic reflex to duplication/deletion analysis
\$1840 full gene sequencing of DNA isolated from tumor in paraffin block, with automatic reflex to duplication/deletion analysis
\$500 screening for deletion/duplication of DNA isolated from blood or tumor
\$340 known familial mutation analysis of DNA isolated from blood
\$340 prenatal diagnosis of sequencing mutation in cells from CVS or amniocytes
\$340 prenatal diagnosis of deletion or duplication in cells from CVS or amniocytes
Samples of maternal and paternal bloods must be submitted with all prenatal testing

CPT codes: Full sequencing from blood or frozen tumor with automatic reflex to duplication/deletion analysis: 83891, 83894x21, 83898x21, 83904x21, 83900, 83901x4, 83909, 83912x2
Full sequencing from tumor in paraffin block with automatic reflex to duplication/deletion analysis: 83891x3, 83894x21, 83898x21, 83904x21, 83900, 83901x4, 83909, 83912x2
Screening for deletion/duplication in blood: 83891, 83900, 83901x5, 83909x2, 83912
Familial mutation from blood: 83891, 83898x6, 83904x6, 83912
Prenatal diagnosis for sequencing mutation or duplication/deletion in CVS or amniocytes: 83891x2, 83898x4, 83904x5, 83912

In cases of patients with unilateral RB tumor and no family history, we recommend testing DNA isolated from the tumor before testing for a mutation in DNA isolated from blood. Please advise the lab if tumor is available but not submitted with blood sample so that we hold the blood until we have received and tested the tumor. If we are not notified in advance that tumor will be coming, we will sequence the blood sample, and if tumor is subsequently sent to be tested, there will be an additional sequencing charge.

Retinoblastoma Testing INSTRUCTIONS FOR SAMPLE SUBMISSION

Documentation: Each sample must be accompanied by:

1. A requisition for DNA analysis completed by the physician, nurse or genetic counselor requesting screening. **Please note: ICD-9 code is required for billing purposes. If ICD-9 code is unknown, please provide patient's clinical symptom(s) or family history that prompted testing.**
2. The patient's pedigree to include three generations, if possible.
3. An informed consent signed by the patient (if under 18 years of age, the parent or guardian should sign) and the professional obtaining the consent. Please have the patient initial at the top of each page and send **all** pages of the consent.
4. A verification of blood tubes form signed by the patient, parent or guardian. The form should be signed at the time of the blood draw.
5. A completed registration form with check, money order, credit card authorization or information for billing the referring institution.

IN THE EVENT THAT ALL PROPERLY COMPLETED FORMS DO NOT ACCOMPANY THE SPECIMEN, YOU WILL BE NOTIFIED, AND TESTING WILL BE HELD UNTIL PAPERWORK IS COMPLETE.

Preparing Sample:

- Obtain 2 EDTA tubes (lavender top) of blood - approx. 4 mL per tube
- Label each tube with the patient's name and date sample was obtained
- Send frozen tumor on dry ice.
- Paraffin tumor block can be cut by our lab and returned after testing is completed.
- If tumor is sent separately from blood sample, please include patient' name.
- We accept banked or recently extracted DNA; please include the concentration.

- For prenatal testing: cultured amniotic fluid or CVS cells, 2 confluent T-25 flasks. Please call the lab prior to sending a prenatal sample. We are often able to offer testing on a direct villi or amnio sample, and we can discuss the requirements with you. 5mL of whole blood from each parent should accompany the prenatal sample.

Shipping Sample: Ship at room temperature via Federal Express or other overnight courier that guarantees AM delivery to arrive Monday-Friday. There is no one in the laboratory evenings and weekends to receive samples. If sample is drawn on a Friday, please refrigerate it until shipment on the following business day.

Shipping Address: Genetic Diagnostic Laboratory
University of Pennsylvania
415 Anatomy-Chemistry Building
3620 Hamilton Walk
Philadelphia, PA 19104

**GENETIC DIAGNOSTIC LABORATORY
UNIVERSITY OF PENNSYLVANIA SCHOOL OF MEDICINE
DEPARTMENT OF GENETICS
Tel: (215) 573-9161 • Fax: (215) 573-5940
CLIA ID: 39D0893887**

REQUEST FOR RETINOBLASTOMA TESTING

PATIENT FIRST NAME _____ PATIENT LAST NAME _____

BIRTH DATE _____ SEX _____ RACE _____ ETHNICITY _____

STREET ADDRESS _____

CITY _____ STATE _____ ZIP _____ HOME PHONE _____

Retinoblastoma Diagnosis: _____ unilateral _____ bilateral

Is there any family history of retinoblastoma? ____ No ____ Yes: Affected relative _____
Please include a family pedigree.

At what age was the patient diagnosed with retinoblastoma? _____

Is tumor being submitted? ____ No ____ Yes, with blood sample ____ Yes, at a later date

Was the tumor removed after radiation or chemotherapy? ____ No ____ Yes: radiation or chemo

REFERRING PHYSICIAN _____ PHONE _____ FAX _____

GENETIC COUNSELOR _____ PHONE _____ FAX _____

EMAIL ADDRESS FOR COUNSELOR OR PHYSICIAN _____
INSTITUTION and
DEPARTMENT _____

STREET ADDRESS _____

CITY _____ STATE _____ ZIP _____ COUNTRY _____

ICD-9 CODE _____

TEST REQUESTED

- _____ Sequencing of the coding regions of the RB gene from frozen tumor and blood with reflex to duplication/deletion analysis
- _____ Sequencing of the coding regions of the RB gene from paraffin tumor and blood with reflex to duplication/deletion analysis
- _____ Sequencing of the coding regions of the RB gene from *blood only* with reflex to duplication/deletion analysis
- _____ Duplication/Deletion analysis on _____ tumor and blood or on _____ blood only
- _____ Screening for known familial mutation in the RB gene _____ sequencing mutation or _____ duplication/deletion
- _____ Prenatal diagnosis of known familial sequencing mutation
- _____ Prenatal diagnosis of known duplication/deletion

Please include a copy of genetic result for affected family member for any familial or prenatal test requests
For all prenatal requests, please call the laboratory to discuss requirements; 5mL of whole blood from both parents should accompany any
prenatal sample for MCC studies.

**GENETIC DIAGNOSTIC LABORATORY
UNIVERSITY OF PENNSYLVANIA SCHOOL OF MEDICINE
DEPARTMENT OF GENETICS
Tel: (215) 573-9161 • Fax: (215) 573-5940**

Patient Registration Form

Please provide the following information. We cannot perform your test without ALL of this information. PLEASE PRINT ALL ANSWERS

PATIENT INFORMATION

LAST FIRST MI

PATINET'S MAIDEN NAME IF PREVIOUS TESTING WAS PERFORMED

Date of Birth: _____

Gender: ___ Male ___ Female

Patient Address: _____
STREET/APT NO.

CITY, STATE, ZIP COUNTRY IF OUTSIDE UNITED STATES

HOME TELEPHONE

REFERRING PHYSICIAN

NAME MD DO OTHER

INSTITUTION

STREET ADDRESS

CITY, STATE, ZIP

TELEPHONE

FAX

PAYMENT OPTIONS (must choose one) [a receipt will be mailed to patient for self-pay options]

_____ I have enclosed a check payable to the "Genetic Diagnostic Laboratory" for \$ _____

_____ Please charge my credit card for the amount of \$ _____
___ VISA ___ Master Card ___ Discover ___ American Express

Card Number: _____ Exp date: _____

Name as it appears on card: _____

_____ I have Pennsylvania Medicaid. A copy of my Medicaid card is attached.

_____ INSTITUTIONAL BILLING: The Institution where my testing originated has agreed to pay all charges for the testing. INCLUDE Billing Address, Person Authorizing Payment, Telephone, and Fax below:

BILLING ADDRESS

BILLING ADDRESS

PERSON AUTHORIZING PAYMENT

PHONE

FAX

Verification of Correctly Identified Blood Tubes

I am a participant in genetic DNA testing.

I have been shown the tubes containing my blood for this genetic testing and my name has been correctly placed on each one of these tubes.

I have signed a copy of the consent form regarding this genetic testing to be sent along with my blood samples. I have been given a copy of the consent form to keep.

Participant Name: _____

Participant/Parent Signature: _____

Date: _____

Initials _____

PROBAND ANALYSIS/Child

**GENETIC DIAGNOSTIC LABORATORY
UNIVERSITY OF PENNSYLVANIA SCHOOL OF MEDICINE
DEPARTMENT OF GENETICS
Tel: (215) 573-9161 • Fax: (215) 573-5940
CLIA ID: 39D0893887**

Informed Consent for Retinoblastoma Testing/CHILD

I, _____, hereby request testing for the **retinoblastoma gene, RB1**, using a DNA-based test. I understand that a sample of my child's blood will be obtained from a vein, a procedure that carries very little risk. In many cases analysis of the tumor provides more complete information about the RB1 mutation. I understand that a tumor sample, if available, can also be sent with my child's blood sample. I understand that the diagnostic samples will be used for the purpose of attempting to determine if my child is a carrier of an altered RB1 gene for this genetic disease.

I understand that:

1. In some cases the DNA test directly detects an abnormality, called a mutation, in the gene and the test is >99% accurate. There is a chance that my child has a mutation which will not be detected by this testing. There is a chance that an alteration of unknown significance may be identified.
2. The DNA analysis performed at the University of Pennsylvania Genetic Diagnostic Laboratory is specific only for this disease and in no way guarantees my child's health.
3. These tests are subject to change periodically to improve or expand the utility of the test. The tests are not considered research but are considered to be the best and newest laboratory service available. This testing is often complex and utilizes specialized materials so that there is a small possibility that the test will not work properly or that an error will occur. There error rate is low, perhaps 1 in 1000 samples. My signature below acknowledges my voluntary participation in this test, but in no way releases the laboratory and staff from their professional and ethical responsibility to me and my child.
4. In some cases it may be possible for the laboratory to reanalyze leftover DNA samples in the future using new and improved methods. However, I understand that the Genetic Diagnostic Laboratory is not a DNA banking facility and my child's DNA sample may not be available for future clinical studies.
5. Because of the complexity of DNA based testing and the important implications of the test results, results will be reported to me only through the physician or genetic counselor who requested the testing. The results are confidential; they will only be released to other medical professionals or other parties with my written consent. Participation in DNA testing is completely voluntary.
6. My child's sample will be used only for the test requested. In some cases, DNA samples may be anonymized (stripped of all identifiers) and used as control samples or in research. Results from such testing can not be attributed to identifiable patients and the results are not reportable.
7. Any blood or tissue specimens obtained for the purposes of this genetic testing become the exclusive property of the Genetic Diagnostic Laboratory. After the specific tests requested have been completed and reported, the Laboratory may dispose of, retain, or preserve these specimens and may use these specimens for research. I understand that my identity will be protected and that research results will not be provided to me or to any other party. If there

Revised 1/27/2009

are new developments in the field, my physician/genetic counselor may be contacted by the Genetic Diagnostic Laboratory staff to offer me the opportunity to have additional clinical testing. If use of this genetic material results in a scientific publication, it will not contain any identifying information.

REQUEST FOR MORE INFORMATION:

I have been assured that my results will not be released to any relative or any other third party without my express written consent. I understand that I may ask more questions about this testing and my results at any time. At the Genetic Diagnostic Laboratory, Susan Walther, MS, CGC (215-573-9161) and Arupa Ganguly, PhD, FACMG (215-898-3122) will be available to answer questions as they arise. I will be given a copy of this consent form to keep.

CONSENT OF PARENT OR GUARDIAN

I have explained to _____ the purpose of this genetic testing, the procedures required and the possible risks and benefits to the best of my ability.

Signature of Professional Obtaining Consent: _____

Print Name: _____ Date: _____

I have read and received a copy of this consent form. I agree to have genetic testing performed for my child and accept the risks. I understand the information provided in this document and I have had the opportunity to ask questions I have about the testing, the procedure, the associate risks and the alternatives.

Signature of Parent/Guardian: _____ Date: _____

Relationship to Child: _____

Print Name of Child: _____ Child's DOB: _____

Initials _____

PROBAND ANALYSIS/Adult

**GENETIC DIAGNOSTIC LABORATORY
UNIVERSITY OF PENNSYLVANIA SCHOOL OF MEDICINE
DEPARTMENT OF GENETICS
Tel: (215) 573-9161 • Fax: (215) 573-5940
CLIA ID: 39D0893887**

Informed Consent for Retinoblastoma Testing/Adult

I, _____, hereby request testing for the **retinoblastoma gene, RB1**, using a DNA-based test. I understand that a sample of my blood will be obtained from a vein, a procedure that carries very little risk. I understand that the diagnostic samples will be used for the purpose of attempting to determine if I (or my fetus) am/is a carrier of an altered RB1 gene for this genetic disease.

I understand that:

1. In some cases the DNA test directly detects an abnormality, called a mutation, in the gene and the test is >99% accurate. There is a chance that I have a mutation which will not be detected by this testing. There is a chance that an alteration of unknown significance may be identified.
2. The DNA analysis performed at the University of Pennsylvania Genetic Diagnostic Laboratory is specific only for this disease and in no way guarantees my health.
3. These tests are subject to change periodically to improve or expand the utility of the test. The tests are not considered research but are considered to be the best and newest laboratory service available. This testing is often complex and utilizes specialized materials so that there is a small possibility that the test will not work properly or that an error will occur. There error rate is low, perhaps 1 in 1000 samples. My signature below acknowledges my voluntary participation in this test, but in no way releases the laboratory and staff from their professional and ethical responsibility to me.
4. In some cases it may be possible for the laboratory to reanalyze leftover DNA samples in the future using new and improved methods. However, I understand that the Genetic Diagnostic Laboratory is not a DNA banking facility and my DNA sample may not be available for future clinical studies.
5. Because of the complexity of DNA based testing and the important implications of the test results, results will be reported to me only through the physician or genetic counselor who requested the testing. The results are confidential; they will only be released to other medical professionals or other parties with my written consent. Participation in DNA testing is completely voluntary.
6. My sample will be used only for the test requested. In some cases, DNA samples may be anonymized (stripped of all identifiers) and used as control samples or in research. Results from such testing can not be attributed to identifiable patients and the results are not reportable.
7. When results of the laboratory testing are available, I will be given the option of postponing or declining disclosure of these results. If I choose to learn the results, I will be given this information as part of a counseling session.
8. Any blood or tissue specimens obtained for the purposes of this genetic testing become the exclusive property of the Genetic Diagnostic Laboratory. After the specific tests requested have been completed and reported, the Laboratory may dispose of, retain, or preserve these specimens and may use these specimens for research. I understand that my identity will be protected and that research results will not be provided to me or to any other party. If there are new developments in the field, my physician/genetic counselor may be contacted by the Genetic Diagnostic Laboratory staff to offer me the opportunity to have

PROBAND SEQUENCING/Adult

additional clinical testing. If use of this genetic material results in a scientific publication, it will not contain any identifying information.

REQUEST FOR MORE INFORMATION:

I have been assured that my results will not be released to any relative or any other third party without my express written consent. I understand that I may ask more questions about this testing and my results at any time. At the Genetic Diagnostic Laboratory, Susan Walther, MS, CGC (215-573-9161) and Arupa Ganguly, PhD, FACMG (215-898-3122) will be available to answer questions as they arise. I will be given a copy of this consent form to keep.

CONSENT OF PATIENT:

I have explained to _____ the purpose of this genetic testing, the procedures required and the possible risks and benefits to the best of my ability.

Signature of Professional Obtaining Consent: _____

Print Name: _____ Date: _____

I have read and received a copy of this consent form. I agree to have genetic testing performed for myself, or my fetus, and accept the risks. I understand the information provided in this document and I have had the opportunity to ask questions I have about the testing, the procedure, the associate risks and the alternatives.

Patient's Printed Name: _____ DOB: _____

Patient's Signature: _____

Date: _____

Initials _____

FAMILIAL MUTATION

**GENETIC DIAGNOSTIC LABORATORY
UNIVERSITY OF PENNSYLVANIA SCHOOL OF MEDICINE
DEPARTMENT OF GENETICS
Tel: (215) 573-9161 • Fax: (215) 573-5940
CLIA ID: 39D0893887**

**INFORMED CONSENT: FAMILIAL MUTATION GENETIC TESTING FOR
RETINOBLASTOMA**

PURPOSE:

I, my child or my fetus, will be tested for the alteration in the retinoblastoma gene which has been identified in one of my family members. I understand that the testing will take approximately 1-3 weeks to complete. The purpose of this genetic testing is to determine whether I, or my child, or my fetus, have an altered retinoblastoma gene that is involved in the early development of retinal tumors. I have had the opportunity to discuss the benefits and risks of retinoblastoma testing for myself, or my child. No information pertaining to the genetic test results will be provided to any of my relatives without my consent.

TESTING PROCEDURE:

Genetic testing requires several teaspoons (2 tubes) of blood (less for a very young child). Before the blood is drawn, I will watch as my name, or my child's name, is written correctly on empty blood tubes and after the blood is drawn I will sign a form indicating that I have positively identified the tubes containing the blood. The blood sent to the laboratory will be divided into two separate samples. The first sample will be used to complete the genetic testing. The second sample will be tested to confirm the diagnosis if the first sample indicates an alteration. There will be no additional charge for the confirmation testing.

If this is a prenatal sample, cells from either a CVS or amniocentesis will be used to isolate fetal DNA for analysis of the DNA mutation found in my family that causes retinoblastoma. In order to confirm that analysis was performed on fetal cells, studies to rule out any maternal cell contamination will also be performed.

RISKS AND DISCOMFORTS:

I understand that there is usually a minimal amount of risk involved in drawing a blood sample. These include pain at the blood drawing site, bleeding, bruising and infection.

If this is a prenatal sample, I have been counseled on the risks of the prenatal procedure, and I understand that the genetic analysis of the retinoblastoma DNA mutation found in my family will determine whether my fetus has inherited the altered gene and for the future risk to develop the retinal tumor associated with the disease.

ALTERNATIVE TO GENETIC TESTING:

I understand that participation in this testing is completely voluntary and will not affect my or my child's medical treatment now or in the future. The alternative is not to undergo testing; in which case I will not learn whether I or my child has an altered form of the retinoblastoma gene. This decision is perfectly acceptable.

RESULTS:

I understand that there are two possible results to this testing:

- 1) I may learn that I, or my child or my fetus, have inherited the clinically significant altered retinoblastoma gene found in my family.
- 2) I may learn that the testing found that I, or my child or my fetus, did not inherit the DNA mutation that is associated with retinoblastoma in my family.

USE OF SPECIMENS:

I understand that any blood or tissue specimens obtained for the purposes of this genetic testing become the exclusive property of the Genetic Diagnostic Laboratory. After the specific tests requested have been completed and reported, the Laboratory may dispose of, retain, or preserve these specimens and may use these specimens for research. I understand that my or my child's identity will be protected and that research results will not be provided to me or to any other party. If there are new developments in the field, my physician/genetic counselor may be contacted by the Genetic Diagnostic Laboratory staff to offer me the opportunity to have additional clinical testing.

REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about this testing and my results at any time. At the Genetic Diagnostic Laboratory, Susan Walther, MS, CGC (215-573-9161) and Arupa Ganguly, PhD, FACMG (215-898-3122) will be available to answer questions as they arise. I will be given a copy of this consent form to keep.

I have explained to _____ the purpose of this genetic testing, the procedures required and the possible risks and benefits to the best of my ability.

Printed Name of Professional Obtaining Consent

Signature of Professional Obtaining Consent

Date

CONSENT OF PATIENT:

I have read and received a copy of this consent form. I agree to have genetic testing and accept the risks. I understand the information provided in this document, and I have had the opportunity to ask questions I might have about the testing, the procedure, the associate risks and the alternatives.

Printed Name of Patient

Patient's DOB

Signature of Patient

Date

CONSENT OF PARENT OR GUARDIAN:

I have read and received a copy of this consent form. I agree to have genetic testing performed for my child and accept the risks. I understand the information provided in this document, and I have had the opportunity to ask questions I might have about the testing, the procedure, the associate risks and the alternatives.

Printed Name of Parent/Guardian

Relationship to Child

Signature of Parent/Guardian

Child's Name

Child's DOB