

## RGSU User Manual

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| DOCUMENT REVIEW HISTORY |  |   |
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| Review date             | Reviewed by                                      | Summary of amendments   |
| Jan 2019                | Zerrin Onadim<br>Elizabeth Price<br>Roopal Patel | Remove CPA logo (now UKAS SOA number 8285)<br>Update TATs table (6.2), and sample collection table (7.1).<br>Add 'Compliments' to section 11. |
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# RETINOBLASTOMA GENETIC SCREENING UNIT USER MANUAL

UKAS accredited Laboratory No 8285  
For details of scope see [www.ukas.com](http://www.ukas.com)

## 1. INTRODUCTION

The Retinoblastoma Genetic Screening Unit (RGSU) is located at the Royal London Hospital and is part of the Pathology Department within Barts Health NHS Trust (UKAS accredited Laboratory No 8285). The Unit also has links with the Barts Health Ophthalmology Department. The laboratory provides a supra-regional genetics service for retinoblastoma patients and their relatives, performing molecular genetic tests for predisposition to retinoblastoma (OMIM reference number 180200). This laboratory does not perform tests for any other disease. Clinical Geneticists, genetic counsellors and physicians (ophthalmologist, oncologist) can request retinoblastoma/*RB1* testing with the patient's or legal guardian's consent. The following details are provided for the users to aid their referral process.

## 2. UNIT CONTACT DETAILS

All samples and correspondence should be addressed to:

Retinoblastoma Genetic Screening Unit  
The Royal London Hospital  
Molecular Pathology Suite  
3<sup>rd</sup> Floor, Pathology & Pharmacy Building  
80 Newark Street,  
Whitechapel  
London  
E1 2ES

Telephone: +44 (0) 20 3246 0265  
+44 (0) 20 3416 5000 x61046  
Fax: +44 (0) 20 3246 0321  
Email: [retinoblastoma@bartshealth.nhs.uk](mailto:retinoblastoma@bartshealth.nhs.uk)  
Website: [www.bartshealth.nhs.uk/Retinoblastoma](http://www.bartshealth.nhs.uk/Retinoblastoma)

## 3. HOURS OF OPERATION

The laboratory is open Monday – Friday, 9.00 am to 5.30 pm excluding bank holidays. No samples will be received outside of these times.

## 4. PERSONNEL CONTACT DETAILS

Dr Zerrin Onadim Head of RGSU/Clinical Scientist  
E-mail: [zerrin.onadim@bartshealth.nhs.uk](mailto:zerrin.onadim@bartshealth.nhs.uk)

Dr Elizabeth Ann Price Clinical Scientist  
E-mail: [elizabeth.price@bartshealth.nhs.uk](mailto:elizabeth.price@bartshealth.nhs.uk)  
All clinical scientists are HCPC registered.

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## 5. OVERVIEW OF RETINOBLASTOMA

The retinoblastoma susceptibility gene, *RB1* (Genbank sequence accession number L11910.1; NCBI RefSeq NM\_000321.2; LRG\_517t1), is located on chromosome 13q14 and is a tumour suppressor gene. Mutations in both copies (alleles) of the *RB1* gene are necessary for the development of retinoblastoma.

In about 50% of affected children, both *RB1* mutations occur in a single retinal cell by chance and cannot be passed to offspring. These children have unilateral (one eye), unifocal (one tumour) disease. In the remaining patients one 'predisposing' mutation is present in germ line cells (sperm or ova) and can be transmitted to offspring. This is genetic/heritable/hereditary retinoblastoma which generally affects both eyes (bilateral disease) when inherited. Somatic and/or germ line mosaicism, where some but not all cells contain the predisposing mutation, is observed in some founders of new mutation lineages.

In the sporadic (no family history) unilateral form of retinoblastoma, it is not possible to know without genetic testing whether the disease will be heritable unless the patient has affected offspring. It is estimated that 15-20% of such sporadic cases do carry germ line mutations. Early identification of these cases requires genetic testing which is most efficiently done using tumour tissue from an enucleated eye.

The spectrum of predisposing *RB1* gene mutations includes large structural changes (about 10-20%) some of which (8%) are also detectable by cytogenetic analysis. Other changes include single base substitutions (about 50-60% of mutations) and small insertions/deletions (about 30%). 60-70% of tumours exhibit loss of heterozygosity (loss of one copy/allele) with a mutation in the remaining copy. Finally, hypermethylation at the *RB1* gene promoter, which inhibits the levels of retinoblastoma protein made by the cell rather than its' function, is observed in around 10% of tumours.

Variable penetrance (the probability that a specific mutation will lead to cancer development) and expressivity (the number of tumours occurring) is a feature of retinoblastoma. Much of this variation is due to the different types of *RB1* mutation. Mutations that result in premature termination are most often associated with almost complete penetrance and bilateral retinoblastoma. However, milder disease with incomplete penetrance and reduced expressivity is usually found in families with missense mutations, some splice site changes, small in-frame deletions and promoter region mutations.

Recently a special form of retinoblastoma with no *RB1* mutations but *MYCN* amplification has been identified but constitutes a minority of cases (~1.5%) and seen only in sporadic unilateral retinoblastoma.

## 6. TESTING

The genetic screening provided is based on current knowledge of the *RB1* gene, but the techniques used are not 100% sensitive and mutation(s) in any given sample could be missed. Therefore, when full mutation screening is negative, it does not always mean that the sample is free of *RB1* mutations. Furthermore, identification of a *RB1* mutation in an individual does not exclude the remote possibility of the presence of a second unidentified *RB1* mutation. It is possible for two relatives to have different *RB1* mutations.

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## 6.1. Tests Offered

The Retinoblastoma Genetic Screening Unit uses a series of different molecular tests to identify *RB1* gene mutations. In cases where mutation identification is not possible, indirect testing using linkage analysis is performed.

### Indirect Testing

Linkage analysis: Intragenic *RB1* and chromosome 13 polymorphisms flanking the *RB1* gene are investigated for linkage exclusion and loss of heterozygosity (LOH) in cases of familial and sporadic retinoblastoma in the absence of mutation information.

| Test                         | Details  |
|------------------------------|--|
| Linkage exclusion testing    | Carried out to exclude siblings of familial and sporadic cases from unnecessary examination under anaesthetic (EUA) if there is no shared <i>RB1</i> allele (25%).   |
| Loss of Heterozygosity (LOH) | LOH (when only the initially mutated allele is left in the tumour - present in 60-70% of Rb tumours) is useful to exclude siblings or offspring of sporadic cases, if the <i>RB1</i> allele that is left in the tumour is not present in the test case. 50% of cases can be excluded when LOH is present. The overall chance of exclusion is 35% when tumour is available and polymorphisms are informative. |

### Direct Testing

*RB1* mutation analysis:

| Test                                     | Details  |
|--|--|
| <i>RB1</i> gene mutation screening (DNA) | HRM analysis can detect 85-95% of point mutations and small deletions/insertions in defined systems. Fluorescent dosage (QF-PCR) analysis can detect 70-80% of heterozygous large abnormalities. MLPA analysis uses SALSA MLPA P047 <i>RB1</i> (MRC-Holland). Around 10% of retinoblastomas exhibit hypermethylation at the <i>RB1</i> promoter. Current mutation detection rates in familial cases, sporadic bilateral cases and in fresh tumours of sporadic unilateral cases are around 95%. Expected mutation detection rates in blood samples of unilateral cases are 12-14%. |
| <i>RB1</i> gene mutation screening (RNA) | If DNA screening does not find a mutation then samples may be requested for RNA screening for deep intronic mutations. Such samples may also be requested for the characterisation of unknown variants (UVs).  |
| Testing for known <i>RB1</i> mutations   | Sequencing, sizing, restriction enzyme digestion, QF-PCR and MLPA analyses and marker typing. Gene carrier testing can be carried out as a pre- (before birth) or peri- (first six weeks of life) natal test. Pre-natal and peri-natal testing is available for previously defined cases with prior notice. NOTE: Linkage analysis may also be used to confirm carrier status.   |

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## 6.2. Specimen Requirements and Turnaround Times

| Test Priority          | Test Type                            | Specimens <sup>#</sup>  | Turnaround times                            |
|------------------------|--------------------------------------|---|---|
| <b>Routine Testing</b> | Peri-natal testing*                  | Cord or peripheral blood  | 5 -14 calendar days                         |
|                        | Linkage exclusion                    | Blood from the patient and relatives  | 21 calendar days                            |
|                        | Exclusion with LOH †                 | Blood and tumour from the patient<br>Blood from relatives                         | 21 calendar days                            |
|                        | Known mutation testing               | Blood from the relative/test case   | 21 calendar days                            |
|                        | <i>RB1</i> mutation screening        | Blood from the patient (and tumour for unilateral sporadic patients if available) | 42 calendar days                            |
| <b>Urgent Testing</b>  | Pre-natal testing with prior notice* | Chorionic villus sample (CVS) DNA and two individual fronds                       | 3 calendar days (5 days for dosage testing) |

<sup>#</sup> DNA is usually extracted from specimens in-house but DNA extracted externally may be acceptable.

<sup>†</sup> Loss of Heterozygosity in the tumour.

\* Maternal Cell Contamination (MCC) testing is carried out on CVS and Cord blood samples. Maternal blood/DNA sample is needed to aid this testing.

## 7. SUBMITTING SAMPLES

A Retinoblastoma Genetic Screening Unit request form, including informed consent for genetic testing should be filled in for each patient (printable copies of this form are available from the website or directly from the unit). Please complete all parts of the form. If sending samples from a family complete a separate form for each family member and state the relation to proband. **Wrong information on relation to proband could result in wrong diagnosis.**

Consent should be gained by the clinician requesting the test. By completing a request form it is deemed that the clinician has gained consent for the appropriate tests and retention of samples. Signed consent is required for testing and storage of tumour material. Prior to signing informed consent for genetic testing, details should be explained to the patient by a genetic counsellor and/or informed medical personnel. The patient(s) should also be informed of the turnaround times of molecular screening tests.

### 7.1. Sample Collection for Molecular Testing

Prepare the sample as detailed in the table below. **All samples should be labelled with at least 2 identifiers (name and date of birth and/or hospital no) or they may be rejected (see 7.4. Unsuitable Samples).**

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| Specimen                 | Sample Collection  | Recommended Transport Conditions   |
|--------------------------|--|--|
| Blood (general testing)  | Collect <b>one tube</b> of 4 -10ml EDTA blood per patient, ensure tube is <b>labelled</b> . Smaller volumes are accepted for infants, but this may adversely affect the quality and range of tests performed.                          | Deliver within 24-48 hours. Store and transport at room temperature.   |
| Blood (RNA testing)      | RNA testing requires <b>prior arrangement</b> with the Unit. Collect blood into a PAXgene collection tube (2.5ml) and into a Lithium heparin (4ml - green top) <b>labelled</b> blood tube. Invert to mix immediately after collection. | Deliver within 24 hours. Store and transport at room temperature. Must arrive in lab Mon-Wed, samples received later in the week will not be accepted. |
| Buccal Cells             | Isohelix collection swabs are available on request from the unit along with sampling instructions.   | Deliver within 24-48 hours. Store and transport at room temperature.   |
| Chorionic Villus Sample  | Prepare DNA from the bulk CVS, plus 2 individual CV fronds (boil preps). Samples should be placed into <b>labelled</b> tubes. If DNA extraction is not possible then contact the Unit for further collection instructions.             | Deliver within 24-48 hours. Store and transport at room temperature.   |
| Fresh Tumour             | A protocol for collection of fresh tumour material from an enucleated eye is available upon request from the unit. These samples are accepted with prior arrangement.  | Deliver within 2 hours. Store and transport on ice.  |
| Paraffin Embedded Tumour | Place 5 cores of tumour material (~ 1mm diameter) into a <b>labelled</b> , sterile plastic tube. A larger single plug of tumour tissue can be extracted using a sterile scalpel. Normal eye tissue should be avoided.                  | Transport at room temperature.   |
| Hair                     | Place a minimum of 10 hairs, with <b>roots attached</b> in a <b>labelled</b> , sterile plastic tube. Instructions are available from the unit on request.  | Transport at room temperature.   |
| DNA                      | Prepare DNA from appropriate/requested material in a <b>labelled</b> tube. Indicate the volume, concentration and <b>method of extraction</b> of DNA sent. Send at least 20 ug DNA with a concentration of at least 100ng/ul.          | Transport at room temperature.   |

Ensure that a current version of the unit **Request Form** is completed for each patient, providing patient, referral, sample and clinical details along with informed consent for genetic testing.

**Current version** forms are available from:

E-mail: [retinoblastoma@bartshealth.nhs.uk](mailto:retinoblastoma@bartshealth.nhs.uk)

Web: [www.bartshealth.nhs.uk/retinoblastoma](http://www.bartshealth.nhs.uk/retinoblastoma)

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## 7.2. Sample Transport

- Place the sample(s) and associated forms in a clear sample transport bag. Place this bag in a transport container, UN3373 compliant as required (all samples except DNA, hair or PST cores). See below for UN3373 instructions. Send the sample to:

Dr Zerrin Onadim (14-60265 or 020 3246 0265)  
Retinoblastoma Genetic Screening Unit  
The Royal London Hospital  
Molecular Pathology Suite  
3rd Floor, Pathology & Pharmacy Building  
80 Newark Street, Whitechapel  
London E1 2ES

- NOTE: If a courier is used for delivery please specify delivery to the MAIN RECEPTION of the Pathology & Pharmacy Building.**
- Notify the Retinoblastoma Genetic Screening Unit that a sample(s) has been sent (see section 2 for contact telephone and email).

## 7.3. Cytogenetic Testing

Patients with retinoblastoma may be referred for cytogenetic analysis to rule out abnormalities involving chromosome 13 (e.g. 13q deletion syndrome).

This testing is **not** carried out by the RGSU and local cytogenetic departments should be contacted to determine sample requirements. Information about the Barts Health Cytogenetic services (part of the SIHMDS services) is available on the trust intranet and external website. Where possible, copies of cytogenetic reports should be forwarded to the Unit.

## 7.4. Unsuitable Samples

The receipt of unsuitable samples may result in sample rejection, re-sampling request, poor sample quality, delays in tests/results and/or possible delivery of wrong diagnosis. In case of doubt please contact the Unit.

The sample(s) may be rejected if there is:

- no label, or it is missing at least two unique identifiers (name and date of birth or name and hospital number)
- an illegible label
- missing request form
- non-matching information on the tube and/or request form
- the wrong type of specimen container and/or it is broken or damaged

## 7.5. UN3373 - Summary of Packing Instruction 650 (PI650)

The packaging needs to be strong enough to withstand shocks normally encountered during transport, transfer and mechanical handling. It should prevent loss of contents due to vibration, or change in temperature, humidity or pressure. There are 3 major components:

- Primary leak-proof container** (i.e. sample/blood tube) – each sample must be individually wrapped to prevent contact if multiple samples are sent in one package.

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- 2) Absorbent material (tissue paper or cotton wool) – enough to absorb the entire contents of the primary containers.
- 3) **Secondary packaging** (plastic tube or a sealed specimen bag) – leak proof to protect the outer packaging. Any paperwork should be outside this container.
- 4) Cushioning material – to secure the secondary packaging within the outer packaging.
- 5) **Outer packaging** – This must be rigid with at least one surface having a minimum dimension of 100x100 mm. They must pass a “drop test” of 1.2 meters when containing primary and secondary packaging. They must be labelled with the following symbol and text:



**BIOLOGICAL SUBSTANCE, CATEGORY B**

The sides of the diamond must be at least 50mm, the width of the line at least 2mm and the text within and adjacent to the diamond at least 6mm high. For full instructions go to [www.hse.gov.uk/biosafety/biologagents.pdf](http://www.hse.gov.uk/biosafety/biologagents.pdf)

## 7.6. Factors affecting test performance/result interpretation

The following key factors may affect the performance of our tests or interpretation of the results achieved:

### Specimen factors:

- Low blood volume (<3ml for full mutation screen)
- Clotted blood sample
- Low quality/quantity of DNA
- Wrong type of specimen container
- Delayed or inappropriate conditions during sample transport to lab (≥4 days from sampling for blood samples)

### Clinical factors:

- Incorrect/inaccurate patient clinical information/diagnosis, family history, relationships
- Multifocal tumours
- Presence of low level mosaic mutations in founder individuals

## 8. RESULTS AND REPORTS

- Qualified staff authorise test results.
- Test results, consisting of a hardcopy report are posted.
- Urgent reports can also be faxed to a secure location upon request.
- No telephone reports are issued.
- No electronic reports are issued.

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- Interim reports are issued in cases where indicative preliminary results are obtained but a delay is expected before the final results will be ready. These reports are clearly marked as ‘interim report’. The subsequent final reports are marked as ‘final report’.
- Amended reports are issued in cases where an error has been discovered in a previous report, or when new information needs to be added. These reports state the amendment to the original, and are marked as ‘amended report’.
- All reports are prepared following the Best Practice Guidelines for Molecular Analysis of Retinoblastoma prepared by the European Molecular Quality Network (EMQN). The mutation nomenclature system is as described by the Human Genome Variation Society (<http://www.HGVS.org/varnomen>).

## 9. CLINICAL ADVICE AND INTERPRETATION

Clinical scientists are available via telephone or email during laboratory opening hours (see sections 2 & 3), for any enquiries regarding sample referral, testing, report interpretation etc. The website also has information for our users (visit [www.bartshealth.nhs.uk/retinoblastoma](http://www.bartshealth.nhs.uk/retinoblastoma)). The Barts Health Trust clinical Retinoblastoma service is also contactable via the website.

## 10. QUALITY AND AUDIT

The Unit participates annually in the external quality assessment scheme organised by the European Molecular Genetics Quality Network (EMQN). Our testing is accredited by the United Kingdom Accreditation Service (UKAS; accreditation number 8285 for Barts Health NHS Trust, Pathology) whose standards are compliant with ISO 15189. Please go to ‘<https://www.ukas.com>’ for the current schedule of accreditation. The Unit submits yearly audit information to the Association for Clinical Genomic Science and the National Commissioning Group (NCG) Department of Health. The laboratory is also a diagnostic member of the UK Genetic Testing Network (UKGTN).

## 11. COMPLIMENTS AND COMPLAINTS

Please provide feedback in writing to the Head of the Unit. Complaints will be dealt with according to Barts Health Complaints procedure.

## 12. PROTECTION OF PERSONAL INFORMATION

All personal information provided will be dealt with in line with Barts Health Data Protection Policy.

### **PLEASE NOTE**

This document is subject to document control and it will be periodically updated. The latest version will be made available without notification at **[www.bartshealth.nhs.uk/retinoblastoma](http://www.bartshealth.nhs.uk/retinoblastoma)** and from the Unit directly.

Where a document is made available electronically, it is the responsibility of the document holder, to ensure that their copy is valid and current at all times.

**The RGSU cannot be held responsible for the validity of this document’s content if accessed from unofficial sites/sources.**

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### 13. GLOSSARY

**Unilateral case:** one eye is affected by retinoblastoma (Rb)

**Bilateral case:** both eyes are affected by Rb. This is hereditary with a 50% chance of being passed to future children (unless there is germ line mosaicism).

**Sporadic (isolated) case:** no other affected individuals in the patient's family i.e. no family history.

**Familial case:** there are other affected individuals (parent, sibling, other relative) in the patient's family i.e. positive family history.

**Heritable / Hereditary / genetic case:** familial cases, isolated bilateral and multifocal unilateral cases are considered to be genetic (i.e. they carry germ line *RB1* mutation and their offspring are at risk of inheriting this mutation). 15-20% of unilateral sporadic cases are genetic cases and can only be proven to be so either by genetic testing, or when they have affected offspring.

**Non-genetic case:** most unilateral sporadic cases with a single focus tumour are non-genetic (that is no mutation in the germ line and their offspring are not at increased risk). Genetic tests can only indicate this if tumour tissue from enucleated eye is available.

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