



## Step 2: Retinoblastoma Genetic Test Sample Requirements

**U.S. insurance patients: a completed *Form 1c: U.S. Insurance Information* must be provided.**

**All submitted samples must be labeled with at least two patient identifiers** (ie. name and date of birth).

### Sample Requirements

#### Bilateral proband or positive family history:

- Blood or DNA from blood.
- Fresh tumor sample is useful if available.

#### Unilateral proband with no family history:

- Both blood sample and fresh or flash-frozen tumor sample are useful for complete analysis.

#### Pre/peri-natal sample options:

- Cultured amniocytes
- Direct amniotic fluid
- CVS tissue
- Cord blood
- DNA extracted from amniocytes
- DNA extracted from CVS

**Maternal blood or DNA from blood** (required for all pre/perinatal samples, to be submitted at same time or prior to procedure).

#### Genetically related family member for known mutation:

- Blood or DNA from blood;
- **Or** buccal swab, (select cases only, contact lab to confirm).

**Provide family member's genetic test report if genetic testing has been performed at a lab other than Impact Genetics**

### Sample Preparation Instructions

#### Blood samples for DNA:

- 10 mls in yellow-topped ACD tubes **or** lavender-topped EDTA tubes (for infants 2-5 mls in pediatric or small tubes) at room temperature, to be received within 5 days after draw.

#### DNA from blood:

- DNA concentration—100 nanograms/microliter.
- DNA quantity—150 microliters.

#### Blood samples for RNA:

- 10 mls in lavender-topped EDTA tubes (for infants 2-5 mls in pediatric or small tubes) on 4° C cool packs, to be received within 48 hours after draw.

#### Buccal swab:

- Kit available (select cases only contact lab directly to request); follow included instructions.

#### Amniocytes:

- Two T25 flasks of cultured amniotic cells **or** DNA extracted from amniotic cells at room temperature (100 nanograms/microliter: minimum 20 microliters).

#### Direct amnio:

- As much volume as possible in conical tube at room temperature, to be received within 48 hours after draw.

#### CVS:

- Send CVS tissue in sterile tissue-culture medium at room temperature **or** DNA extracted from CVS tissue at room temperature (100 nanograms/microliter: minimum 20 microliters).

#### Fresh tumor in medium

**(instructions for enucleating surgeon or pathology lab):**

After removal of an eye containing retinoblastoma,

- Cut off optic nerve and retain as separate specimen for pathology lab.
- Open globe by pupillary-optic nerve section as in routine eye pathology.
- Excise or scoop the bulk of the tumor from inside the eye, leaving tumor-optic nerve and tumor-choroidal relationship undisturbed for pathological evaluation.
- Place fresh retinoblastoma tumor in a sterile tissue culture media, such as RPMI or Alpha MEM with antibiotic added (100U/ml penicillin and 100ug/ml streptomycin) and seal container securely with parafilm.
- Tumor may be kept at room temperature until it is ready to be shipped. **DO NOT FREEZE** tumor in tissue culture.
- Retain the remainder of the eye for pathology.
- Send tumor sample at room temperature.

#### Frozen tumor sample preparation:

- Flash freeze tumor sample and ship on enough dry ice to keep frozen for maximum transit time.
- Detailed tumor extraction information available on our website:

<http://impactgenetics.com/testing-services/retinoblastoma/info-for-rb-clinicians/>

### Sample Identification

- Label each sample **with at least two** patient identifiers (e.g. name and date of birth).