

*BloodCenter of Wisconsin  
Diagnostic Laboratories offers Erythroid Chimerism testing.*

## BACKGROUND:

Sickle cell disease (SCD) is a common and severe autosomal recessive disorder caused by a missense mutation in the hemoglobin gene (HBB) resulting in hemoglobin S. Nonmyeloablative allogeneic hematopoietic stem cell transplantation (HSCT) is increasingly being used to treat severely affected patients. Mixed chimerism is observed in a large proportion of patients after HSCT. Most patients transition to complete donor chimerism. Some patients have long-term persistent mixed chimerism without need of red cell support even with a low percentage of donor-derived nucleated cells since an adequate amount of the normal HBB transcript, HbA, is being made.

Several studies have demonstrated that chimerism measured in the nucleated cell compartment does not always reflect the engraftment in the erythroid lineage.<sup>1,2</sup> Assessment of the chimerism in the erythroid lineage may be a better indicator of donor erythropoiesis. As red cells do not contain DNA, chimerism in the erythroid compartment can be monitored using quantitative measurements of HbA and HbS transcripts produced from the hemoglobin gene (HBB) expressed in red cell progenitors.

## REASONS FOR REFERRAL:

- Monitor erythroid lineage chimerism in patients with sickle cell disease following allogeneic bone marrow transplantation
- Monitor effects of post-transplant therapies.

## METHOD:

Reverse transcription of total RNA followed by digital droplet PCR with fluorescently-labeled hydrolysis probes specific for the HBB gene HbA and HbS transcripts. The percent chimerism is calculated taking into account the genotype of the donor (AA or AS).

## REPORTABLE RANGE:

Results are reported as 0%-100% HbA, 0-100% HbS, 0-100% Donor and 0-100% Recipient.

#### LIMITATIONS:

- Assay is limited to SCD patients with SS genotype transplanted with a donor whose genotype is AA or AS.
- Genotype of the donor is required to accurately quantify chimerism.
- Assay sensitivity:
  - o 1% HbA and 1% HbS transcript
  - o 1% Donor and 1% Recipient when the donor genotype is AA
  - o 1% Donor and ~5% Recipient when the donor genotype is AS
- Specificity/sensitivity may be affected by rare polymorphisms in the PCR priming or probe binding sites.

#### SPECIMEN REQUIREMENTS:

3-5 ml EDTA Bone Marrow (lavender top) or 10 ml EDTA Whole Blood (lavender top)

Minimum/Pediatric volume: 1 ml

*Sample must be received within 48 hours of collection.*

*Indicate on specimen tube and requisition whether sample is whole blood or bone marrow.*

#### SHIPPING REQUIREMENTS:

Place the room temperature specimen and requisition in plastic bags, seal and insert in a Styrofoam container. Seal the Styrofoam container, place in a sturdy cardboard box and tape securely. Ship the package in compliance with your overnight carrier guidelines. Package must not arrive on weekends and holidays. Address package to:

Client Services/Molecular Diagnostics Laboratory  
BloodCenter of Wisconsin  
638 N. 18th Street  
Milwaukee, WI 53233  
800-245-3117, ext. 6250

TURNAROUND TIME: 3-6 days

CPT CODES: 81479

#### REFERENCES:

1. Wu, Catherine, et al. Molecular assessment of erythroid lineage chimerism following nonmyeloablative allogeneic stem cell transplantation. *Experimental Hematology* (2003) 31: 924-933.
2. Andreanni, Marco et al. Quantitatively different red cell/ nucleated cell chimerism in patient with long-term, persistent hematopoietic mixed chimerism after bone marrow transplantation for thalassemia major or sickle cell disease. *Haematologica* (2011) 96:128-133.