

BACKGROUND:

Important clinical events in allogeneic bone marrow transplantation such as engraftment, relapse, and the effects of post-transplant therapies can be monitored on a molecular level by detecting genetic differences between recipient and donor. Our test takes advantage of highly polymorphic repetitive loci called VNTRs (Variable Number of Tandem Repeats) or STRs (Small Tandem Repeats). Test results are used to establish relative amounts of recipient and donor present in the post-transplant period.

Enriched cell populations can be used to monitor engraftment patients following hematopoietic stem cell transplant. Testing for the following cell types is available through BloodCenter of Wisconsin:

Cell Marker	Cell Type
CD3	T cells
CD19	B cells
CD33	Myeloid cells
CD56	NK cells

METHOD:

Chimerism testing is performed using two multiplex amplification reactions of eight total VNTR or STR loci. Amplification results in fluorescently-labeled products that uniquely identify recipient and donor alleles. The fluorescence of recipient and donor alleles is used to calculate the percent chimerism. Using the most appropriate markers, the average % recipient and % donor are reported rounded to the nearest integer. The sensitivity, the lowest detectable amount of chimerism, is also reported for each sample. This test is performed in two parts: 1) a pre-transplant analysis to determine which loci can be used to uniquely identify recipient and donor alleles; and 2) post-transplant analysis to establish relative amounts of recipient and donor DNA.

Cell populations are prepared using monoclonal antibody coupled magnetic microbeads. Flow cytometry is used to analyze purity and population/yield of enriched cells. The enriched cells are then used in chimerism testing.

LIMITATIONS:

- An informative locus must be present for chimerism testing on post-transplant samples.
- The sensitivity for recipient and donor alleles is typically 1-2%. The sensitivity is decreased in samples with very low cell counts. The sensitivity may also be affected by sub-optimal allele combinations in the recipient and donor.

REASONS FOR REFERRAL:

- Monitor hematopoietic reconstitution following allogeneic bone marrow transplantation.
- Monitor effects of post-transplant therapies.
- Monitor minimal residual disease.
- Measure chimerism in cellular subpopulations.

TESTING SCHEDULE:

Turnaround time is 7 days for pre-transplant analysis and 3-6 days for post-transplant analysis. STAT testing turnaround time is 48 hours for post-transplant analysis, and 72 hours for post-transplant analysis when cell enrichment is requested.

SPECIMEN REQUIREMENTS:

Pre-transplant

(Recipient and Donor)

5 ml EDTA whole blood:

- Recommended number of cells: 25 million or more
- Minimum number of white cells: 2 million white cells
- Other anticoagulants accepted

Cells/Buccal swabs:

- Contact Molecular Diagnostics Laboratory for instruction

Post-transplant

Without Cell Enrichment

(Recipient)

5 ml EDTA whole blood or bone marrow:

- Minimum: 2 million cells
- Other anticoagulants accepted

Cells:

- Contact Molecular Diagnostics Laboratory for instruction

Post-transplant

With Cell Enrichment

(Recipient)

Available targets include: CD3, CD19, CD33, CD56

14 ml Na Heparin whole blood per cell population sort:

- Minimum: 2 million cells
- EDTA (lavender top), ACDA (yellow top) whole blood, or bone marrow acceptable.
- **Sample must be received within 24 hours of draw**
- Samples may be drawn Monday - Thursday
- Enrichment is performed Tuesday - Friday

SHIPPING REQUIREMENTS:

Ship at room temperature. Place specimen and Molecular Diagnostics Laboratory test requisition into plastic bags and seal. Place in a Styrofoam container and seal; and then into a sturdy cardboard box and tape securely. Ship the package in compliance with your overnight carrier guidelines. **Whole blood or bone marrow for Cell Enrichment must be received at room temperature Monday through Friday only.**

Label with the following address:

Client Services/Molecular Diagnostics Laboratory
BloodCenter of Wisconsin
638 N. 18th Street
Milwaukee, Wisconsin 53233-2121

CPT CODES:

Pre-Transplant	CPT Codes: 81265
Cell Enrichment	CPT Codes: 81268 per marker
Post-Transplant – Each Sample	CPT Codes: 81267

REFERENCES:

1. Luhm RA, Bellissimo DB, Uzgiris AJ, Drobyski WR, Hessner MJ. (2000) Molecular Diagnostics 5:129-138.
2. Bader P, Niethammer D, Willasch A, Kreyenberg H, Klingebiel T (2005) Bone Marrow Transplant. 35:107-119.
3. Kahn F, Agarwal A, Agrawal S, (2004) Bone Marrow Transplant. 34:1-12.

PO Box 2178
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Location/Sample Deliveries:

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