

Chimerism Testing for Engraftment

Versiti offers rapid and highly sensitive chimerism testing for post-transplant monitoring^{2,3}. 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 genetic loci called Variable Number of Tandem Repeats (VNTR) and Short Tandem Repeats (STR). Test results are used to monitor the relative amounts of recipient and donor cells present after transplant¹.

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

Cell Marker	Cell Subset
CD3	T cells
CD19	B cells
CD33 (incl. CD66b)	Myeloid cells
CD56	Natural Killer cells

Indications for testing:

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

Test method:

Chimerism testing is performed using eight total VNTR or STR loci that are used to uniquely identify recipient and donor cells in the 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. PCR amplification and fragment analysis by electrophoresis of the appropriate informative loci identified in pre-transplant samples enables relative quantification of recipient and donor cells. The average % recipient and % donor values are reported rounded to the nearest integer. The sensitivity, the lowest detectable amount of chimerism, is also reported for each sample.

Chimerism can be done from whole blood, bone marrow, and enriched cell subsets that are prepared using monoclonal antibody coupled magnetic microbeads. The enriched cells are then used in chimerism testing to provide deeper analysis of transplant dynamics. Flow cytometry is used to analyze purity and population/yield of enriched cells.

Assay sensitivity and limitations:

The sensitivity for recipient and donor alleles is typically 1-2% dependent on cell counts in the sample. The sensitivity is decreased in samples with cell counts below 2 million white cells. The sensitivity may also be affected by sub-optimal allele combinations in the recipient and donor.

Pre-transplant analysis must be performed on donor and recipient samples before post-transplant analysis can be done.



Reporting of results:

The average % recipient and % donor values from the informative loci are reported rounded to the nearest integer. The sensitivity, the lowest detectable amount of chimerism, is also reported for each sample. Purity of the cell subset is reported if enrichment is ordered.

Specimen requirements:

Pre-transplant Recipient and Donor:

- 5 ml whole blood
- Collection tube anticoagulants EDTA, Na Heparin, or ACDA
- 4-8 Buccal swabs each

Post-transplant (Recipient):

- 5 ml whole blood or marrow
- Collection tube anticoagulants EDTA, Na Heparin, or ACDA
- Cells: Contact Laboratory for requirements

Post-transplant with cell enrichment: (Recipient)

- **Samples must be received within 24 hours of collection**
- Collection tube anticoagulants EDTA, Na Heparin, or ACDA
- Samples for cell enrichment & chimerism can be received Tuesday–Friday

Volume Requirements

Cell Enrichment	Required Volume Blood or Marrow
CD3 or CD33	4ml
CD3 & CD33	8ml
CD19 or CD56	8ml
CD19 & CD56	16ml
CD3 & CD33 & CD56	16ml



SHIP

Shipping requirements:

Ship at room temperature. Place specimen and 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 Tuesday through Friday only.

Label with the following address:

Versiti Client Services
Histocompatibility Laboratory
638 N. 18th Street
Milwaukee, Wisconsin 53233-2121



ORDER

Required forms:

Versiti Wisconsin Requisition

CPT Codes/Billing/ Turnaround time:

CPT and Order Codes are provided for reference purposes only and are subject to change. They are not intended as a guide for internal billing procedures. Institution is solely responsible for identification of correct billing codes.

CPT codes:

Pre-Transplant Chimerism Analysis: CPT 81265

Post-Transplant Chimerism Analysis (Blood or Bone Marrow): CPT 81267

Cell enrichment + Chimerism Analysis: CPT 81268 per marker

Turnaround Time:

3-6 days for pre-transplant and post-transplant analysis from total blood or marrow.

4-7 days for cell enrichments with chimerism analysis.

STAT testing is 1-2 days for blood or bone marrow and up to 3 days for cell enrichments with chimerism analysis.

References:

1. Luhm RA, Bellissimo DB, Uzgiris AJ, Drobyski WR, Hessner MJ. Quantitative evaluation of post—bone marrow transplant engraftment status using fluorescent-labeled variable number of tandem repeats. (2000) *Molecular Diagnostics* 5:129-138.
2. Bader P, Niethammer D, Willasch A, Kreyenberg H, Klingebiel T. How and when should we monitor chimerism after allogeneic stem cell transplantation? (2005) *Bone Marrow Transplant*. 35:107-119.
3. Kahn F, Agarwal A, Agrawal S. Significance of chimerism in hematopoietic stem cell transplantation: new variations on an old theme. (2004) *Bone Marrow Transplant*. 34:1-12.