For hematopoietic progenitor cell engraftment assays, internal controls are used to determine appropriate genotypes or at least to distinguish patient from donor(s) with each run.

NOTE: There must be criteria for the acceptance and rejection of the amplification of a particular genetic locus or individual sample.

HSC.38180 Preferential Amplification **Phase II**

Reactions are optimized to avoid preferential amplification. The minimum amount of DNA is determined to obtain optimal sensitivity.

NOTE: Method validation must include a dilution study to evaluate the concentration of DNA to determine minimum sensitivity of the assay.

HSC.38190 Cell Subset Purity **Phase II**

If cell subset enrichment is performed, the patient report includes the actual or approximate purity of the cell subset.

NOTE: The determination of the actual or approximate purity of the cell subset does not imply that the purity determined in validation studies can be used without further evaluation. An actual measurement may be performed at the time of sample testing. Some isolation methods and cell subpopulations (eg, CD56) may not produce enough cells to test purity and run the monitoring engraftment test. At a minimum, the purity can be determined for each lot of reagent used to isolate the cell subset and then be reported as an approximate purity for that specific lot.

HSC.38200 Hematopoietic Progenitor Cell Engraftment Testing **Phase II**

For hematopoietic progenitor cell engraftment, samples from pre-transplant patient (recipient), pre-transplant donor(s), post-transplant patient, and an appropriate control are analyzed concurrently.

NOTE: Previously generated data from pre-transplant specimens may be used to compare to post-transplant results if a validated system is used to identify and link the appropriate data files for concurrent analysis.

Evidence of Compliance:
- ✓ Records of HPC testing

HSC.38205 Engraftment Analysis **Phase II**

Prior to evaluating post engraftment specimens, the laboratory evaluates a specimen from the donor(s) and a pre-transplant specimen from the patient to determine the number of informative loci to test in order to meet the minimum number of loci needed for calculations.

Evidence of Compliance:
- ✓ Records of hematopoietic progenitor cell engraftment testing

HSC.38208 Preferential Allele Amplification **Phase II**

Preferential allele amplification is considered in the interpretation of hematopoietic progenitor cell engraftment tests.

HSC.38220 Minimal Number of Informative Loci **Phase II**

For hematopoietic progenitor cell engraftment testing, a minimum of three informative loci are routinely used in the calculations.

NOTE: There are exceptions to this rule. Informative loci refer to loci that can distinguish between donor(s) and recipient. An exception for the number of informative loci used may occur in syngeneic twins (donor(s) and recipient) and rarely in closely related donor(s) and recipient.

***REVISED** 12/26/2024*

HSC.38658 Result Reporting **Phase II**

For hematopoietic progenitor cell engraftment, the final report includes an appropriate summary of the methods, the loci tested, the number of informative loci used, the percent donor cells, an indication of any trace cells, and the sensitivity of the assay.

NOTE: For hematopoietic progenitor cell engraftment, when performing testing by next generation sequencing (NGS), the loci tested are not required to be listed on the report.

REFERENCES
1) Clark JR, Scott SD, Jack AL, et al. Monitoring of chimerism following allogeneic haematopoietic stem cell transplantation (HSCT): Technical recommendations for the use of short tandem repeat (STR) based techniques, on behalf of the United Kingdom National External Quality Assessment Service for Leucocyte Immunophenotyping Chimerism Working Group. *Br J Haematol.* 2015;168(1):26-37.
2) Organ Procurement and Transplantation Network (OPTN) Bylaws. Appendix C. Membership Requirements for Histocompatibility Laboratories. US Department of Health and Human Services. Organ Procurement and Transplantation Network (OPTN) Bylaws. Appendix C. Membership Requirements for Histocompatibility Laboratories. US Department of Health and Human Services. December 5, 2022.

ADDITIONAL MOLECULAR TESTING METHODS

HSC.38690 ABO and RhD Typing by Molecular Methods **Phase II**

ABO and RhD typing performed by molecular methods is used for presumptive ABO and RhD typing only. Donor-recipient ABO and RhD typing for transfusion and transplant compatibility evaluations is performed using FDA-cleared or approved serologic methods.

*NOTE: Transplant donor registries often collect samples from potential donors using buccal swabs or saliva. These samples cannot be used for traditional serological ABO/RhD blood group typing because fresh intact red blood cells (RBCs) are not available. Molecular ABO and RhD typing may be performed to predict the presumptive ABO and RhD phenotype to aid in finding an appropriate donor. Because the ABO and Rh genes are complex, prediction of ABO and Rh phenotype by molecular methods is currently used in immunohematology red cell reference laboratories that focus on blood typing complications, for research, or for providing **preliminary** information that can be confirmed by FDA-cleared or approved methods.*

The use of molecular based screening assays is not acceptable for ABO and RhD blood type assignment for the purposes of transfusion or transplantation. ABO and RhD typing by FDA-cleared or approved serologic methods must be used for the purpose of transfusion or donor and recipient ABO and RhD typing for transplantation.

Evidence of Compliance:

✓ Donor-recipient compatibility records with serologic ABO and RhD typing results