Assessment of Chimerism Using Two Next-Generation Sequencing (NGS) Based Methods

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ABSTRACT

BACKGROUND

Short-tandem repeat (STR)-based chimerism testing has been the gold standard for assessment of engraftment after allogeneic hematopoietic stem cell transplant (allo-HSCT). In this study, two next generation sequencing (NGS)-based multiplex methods using either insertions/deletions (indels) or single nucleotide polymorphisms (SNPs) were compared.

METHODS

DNA extracted from peripheral blood of 10 healthy volunteers was used to create a broad range of pre-defined mixed chimerism percentages and establish a reportable range. Extracted DNA from 18 post-HCT samples and 15 proficiency samples were tested by both NGS-based chimerism methods and compared to the reportable range to assess concordance.

RESULTS

A reportable range of 0.1-50% recipient DNA was determined based on the pre-defined chimerism mixtures tested in triplicate to assess linearity, precision, and sensitivity (Table 1). The limit of quantitation (LoQ) was determined to be 0.1% per the lowest concentration of recipient DNA to meet the pre-defined acceptance criteria (CV < 25%). Comparison of results from the 33 tested samples demonstrated strong correlation between both methods (y=1.035x + 1.6836, R^2=0.9925, R=0.9963).

CONCLUSIONS

A comparison of results shows that both indels and SNPs can quantitate percent recipient DNA with high levels of sensitivity in patients with mixed chimerism. Based on precision and linearity testing, both methods achieve lower limits of detection without sacrificing the level of sensitivity seen with traditional STR testing. Using the established reportable range, both methods show concordance.

METHODS

PROCEDURAL FACTORS

DNA was extracted from whole blood using the EZ1&2 DNA Blood 350 µl Kit (Qiagen, Valencia CA) according manufacturer instructions. Samples were tested by both indels (Devyser, Stockholm, Sweden) and SNP (CareDx, Brisbane, CA) based NGS methods based on manufacturer instructions. Sequencing was performed using the MiSeq instrument and consumables (Illumina, San Diego, CA). Sample analysis was completed using manufacturer provided software and laboratory pre-defined criteria.

INFORMATIVE MARKER IDENTIFICATION

Insertion and Deletions (Indels)

Multiplex PCR amplification of 24 genetic markers across 16 chromosomal locations. Recipient and donor sequences are each compared to a reference genome; genotype is based on if the sequence matches the reference (+) or an alternative sequence (-).

Marker Type	Recipient	Donor
Homozygous Informative	+/+	-/-
	-/-	+/+
Heterozygous Informative	+/-	-/-
	+/-	+/+
Uninformative	+/+	+/+
	+/-	+/-

Single Nucleotide Polymorphisms (SNPs)

Multiplex PCR amplification of 202 targeted SNPs across 22 autosomal chromosomes. Recipient and donor sequences are compared to each other to identify informative markers based on sequence variations at SNP locations.

Marker Type	Recipient	Donor
Recipient Heterozygous Informative	G/A	G/G
Donor Heterozygous Informative	G/G	G/A
Homozygous Informative	G/G	A/A
Uninformative	G/A	G/A

RESULTS

TABLE 1: DETERMINATION OF THE LIMIT OF QUANTITATION

Pre-defined mixed percent	Intra-assay		Inter-assay	
% Recipient Chimerism	Mean	CV	Mean	CV
50	51.9	1.3	51.5	4.6
25	22.8	1.97	22.8	9.5
10	8.6	4	8.6	12.3
1	0.9	7.8	0.91	15.3
0.5	0.49	6.9	0.48	10.1
0.3	0.28	13.8	0.27	21.5
0.2	0.19	11.3	0.19	22.1
0.1	0.09	20.6	0.09	24.7
0.01	0.02	49.4	0.02	52.7
LoQ	**0.1%**		**0.1%**	

FIGURE 1: COMPARISON OF %RECIPIENT CHIMERISM BY TEST METHOD

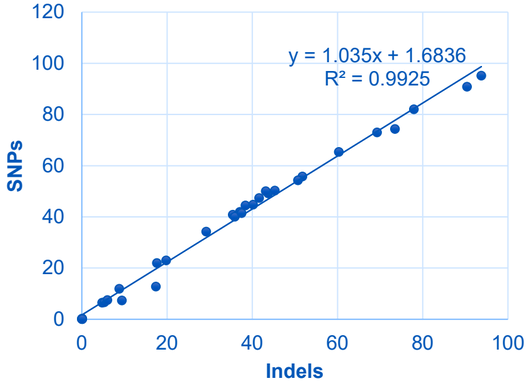

TABLE 2: NUMBER OF INFORMATIVE MARKERS USED FOR ANALYSIS FOR EACH DONOR/RECIPIENT PAIR

Donor Recipient Pair	Indels		SNPs*	
	n	(%)	n	(%)
1	5	(21%)	125	(62%)
2	9	(38%)	133	(66%)
3	9	(38%)	124	(61%)
4	7	(29%)	86	(43%)
5	5	(21%)	85	(42%)
6	6	(25%)	129	(64%)

* Indicates the number of markers analyzed for targeted analysis

LIMITATIONS

Both assays noted that testing should not be performed for patients that met the following criteria:

- HCT transplant with 3 or more donors
- HCT transplant from an identical (monozygotic) twin
- Received a blood transfusion in the last 30 days, with non-leukoreduced cells in the past 30 days