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PERFORMANCE PARAMETERS OF AN NGS PRODUCT FOR CHIMERISM MONITORING

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BACKGROUND

Relapse of the underlying malignant disease is a major complication after hematopoietic stem cell transplantation (HSCT). To detect relapse as early as possible, different methods to determine chimerism have been developed and are currently used in laboratories world-wide. The aim of this study was to evaluate the performance of a novel NGS-based chimerism method and compare it to previously established molecular techniques for chimerism analysis.

MATERIALS AND METHOD

Patient (screened) and artificial samples of chimerism with increasing amounts of recipient DNA were analysed and compared using real-time PCR of insertions/deletions (indels), fragment analysis of short-tandem repeats (STR), and NGS of indels.

The Devyser Chimerism NGS kit for detection of mixed chimerism employs highly informative genetic markers distributed through the human genome (Fig.1). The markers are population independent (Fig.2).

The kit enables screening of donors and recipients prior to HSCT, as well as quantitative determination of mixed chimerism in the recipient following HSCT.

Figure 1. Distribution of genetic markers in Devyser Chimerism NGS kit

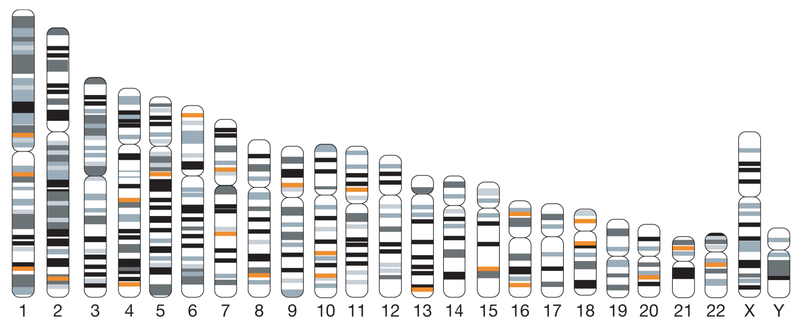
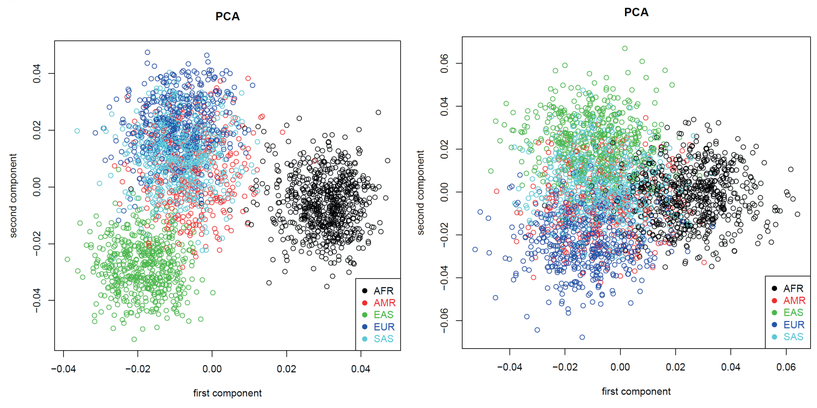


Fig 2. PCA plots of random markers and Devyser Chimerisms genetic markers



PCA for 48 random markers:
- These markers capture the variation between populations
- Samples from the same populations (i.e. related samples) cannot be distinguished.

Parameter results:
- High FST-index and LD

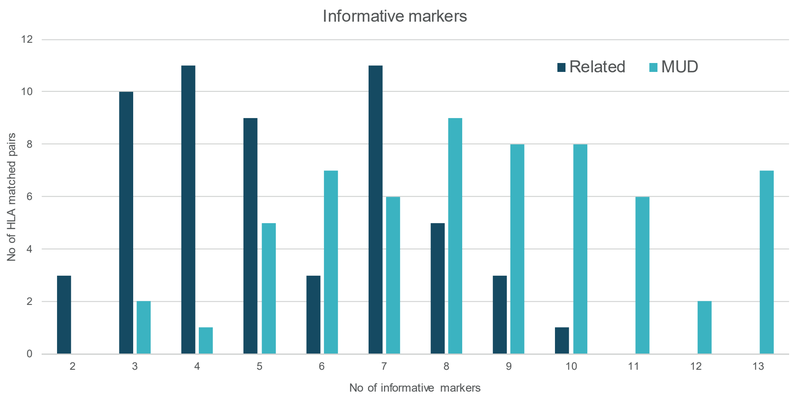
PCA for Devyser Chimerism markers:
- No clear population structure meaning that the markers are able to distinguish persons from the same population/ family

Parameter results:
- Low FST-index and LD

RESULTS

Devyser Chimerism exhibited at least three (average eight) and at least two (average five) informative genetic markers (indels), suitable for monitoring mixed chimerism of patients with their corresponding matched unrelated (60) or related (56) donor samples (Fig. 3).

Figure 3. Number of informative markers in related HLA matched pairs and unrelated HLA matched pairs (MUD)



Each marker in Devyser Chimerism displayed a high accuracy across a broad spectrum of chimerism. Nine informative markers were used, and the highest CV% was 30% at 0,1% Chimerism (Fig. 4 and 5).

Figure 4. Level of Chimerism in each marker (high level). Red line: Theoretical target Chimerism, dotted lines: +/- 5 and 10%

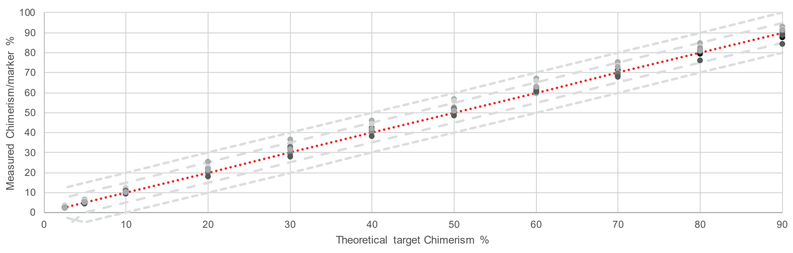
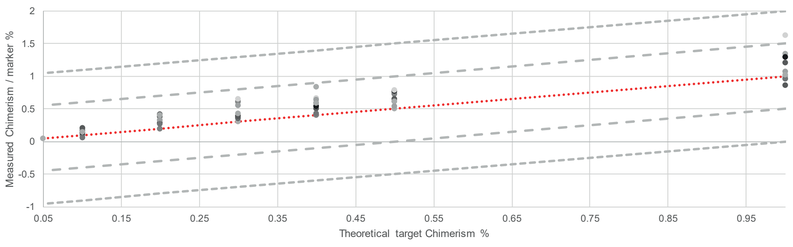


Figure 5. Level of Chimerism in each marker (low level). Red line: Theoretical target Chimerism, dotted lines: +/- 0,5 and 1%



When STR analysis was compared to the NGS kit using artificial chimerism samples with a decreasing DNA amount in the PCR, the STR analysis failed due to allele drop-out, while the NGS kit could detect the chimerism down to 0,9 ng DNA in PCR (Table 1). The CV% increased when the DNA amount decreased.

Table 1. Devyser Chimerism compared to STR analysis with decreasing amount of DNA input

	DNA concentration input (ng/PCR)									
20% MC	15 ng		7,5 ng		3,75 ng		1,85 ng		0,9 ng	
	%	**CV%**	**%**	**CV%**	**%**	**CV%**	**%**	**CV%**	**%**	**CV%**
Devyser Chimerism	20%	1,00%	20%	0,50%	20%	8,50%	18%	5,90%	19%	6,30%
STR analysis	37%	3,00%	36%	0,80%	41%	9,90%	35%	9,90%	ND	ND
5% MC	15 ng		7,5 ng		3,75 ng		1,85 ng		0,9 ng	
	%	**CV%**	**%**	**CV%**	**%**	**CV%**	**%**	**CV%**	**%**	**CV%**
Devyser Chimerism	5%	1,00%	5%	4,30%	5%	1,80%	5%	8,50%	3%	31%
STR analysis	11%	7,10%	10%	6,70%	ND	ND	ND	ND	ND	ND

Real-time PCR displays excellent sensitivity down to 0,01% chimerism, but poor precision above 20%, fragment analysis exhibits good precision with limited sensitivity (> 2,5%). In contrast, NGS chimerism detection demonstrates good sensitivity, with a limit of detection (LOD) of 0,1% chimerism, and precision throughout the whole spectrum of patient/donor mixed chimerism (Fig. 6 and 7).

Figure 6. Devyser Chimerism compared to STR and Real-time PCR analysis (high level) Artificial dilution series.

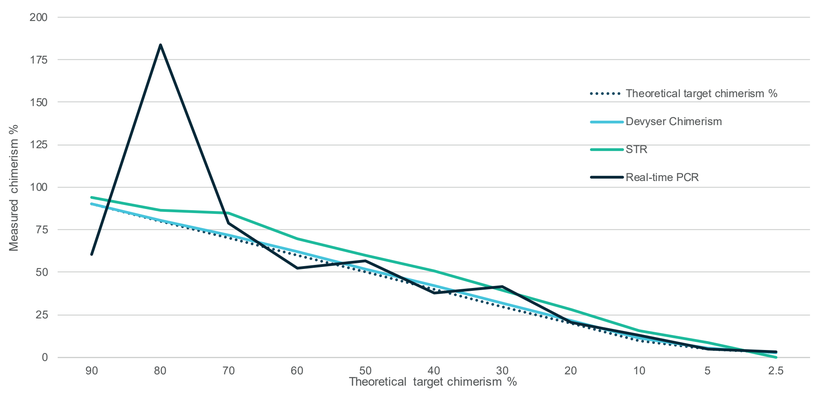
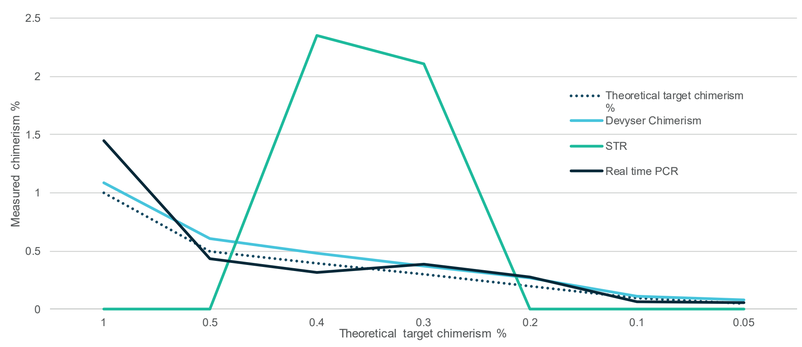


Figure 7. Devyser Chimerism compared to STR and Real-time PCR analysis (low level). STR analysis failed at 1 and 0,5%



CONCLUSION

These results show that although all evaluated techniques are suitable for monitoring patient/donor chimerism after allogeneic hematopoietic stem cell transplantation (HSCT), only the NGS chimerism product exhibits high sensitivity (LOD 0,1 %) and a broad dynamic range (detection range 0,1-100%) with good precision and accuracy throughout the whole spectrum of chimerism (% patient/donor). In addition, the NGS chimerism product employs non-population dependent, highly informative genetic markers which provide stable resolution power and are therefore suitable for monitoring chimerism.