

personalizing diagnostics

Chimerism monitoring by qPCR, a robust performance from 0.05% to 100%

Introduction

Investigating the chimeric status of a recipient after stem cell transplantation is required for the detection of adverse transplant events. The use of qPCR for chimeric monitoring allows for a quick and easy measurement of recipient cells in a donor background, with a sensitivity that can be as low as 0.05%. In comparison to STR analysis with a sensitivity of 1-5%, this is a substantial improvement.

Next, the deviations between the percentage of the individual assay measurements and the mean percentage measured for each sample were determined. This deviation was then plotted against the mean percentage as shown in Figure 2. From this figure it can be observed that there is indeed a higher absolute deviation at higher percentages in the data.

Conclusions

Although the absolute deviation between measurements may be larger at higher percentages, the relative error does not change significantly. We can therefore conclude that the qPCR technique applied for chimerism monitoring with KMRtype/KMRtrack is able to accurately quantify genetic material in a chimeric mixture, not only in the lower levels but confidently from 0.05% to 100%.

The general presumption however, is that qPCR is most accurate in its detection of percentages below 20-30% and less accurate for higher percentages. Here, we provide evidence that qPCR data can be trusted regardless the level of measured percentage.

Materials & Methods

We have collected data of 19 studies, spanning 6 years, from the UK NEQAS 'Post-Stem Cell Transplant Chimerism Monitoring' program. Each study consisted of four samples; one from the recipient, one from the donor and two samples with a chimeric status representing two time points after transplantation.

The recipient and donor samples were genotyped applying the KMRtype workflow (GenDx) consisting of 39 markers in total with diverse locations across the genome. The chimeric mixtures were then examined with all found informative markers for both recipient and donor applying the KMRtrack workflow (GenDx), resulting in 498 individual assay measurements in total. Tests were performed on either a ViiA7 or QuantStudio 6 qPCR system (Thermo Fisher).

Collected data was compared with the results reported by UK NEQAS, which

Finally, the relative error was calculated as described and plotted against the same mean percentage (Figure 3). It can be observed that the relative error is not changing significantly throughout the whole range.



Figure 1. (A) The percentage obtained by GenDx for each measurement, plotted against the mean percentage from all participating sites of UK NEQAS in a linear scale. A linear trend is observed with R2 being 0.9861. (B) Same figure with logarithmic scale.

are based on the results from all participating labs in the program. From this comparison the absolute deviation in percentage and relative error of the qPCR assay were obtained. The relative error was determined with the following calculation:

Relative error = (percentage assay – mean percentage) / mean percentage

Results

The chimerism percentages obtained covered a full range of chimeric status from 0 to 100%.

The percentages obtained at GenDx for each measurement were compared to the mean percentage from the participating sites of UK NEQAS (Figure 1, with A representing the data in a linear scale and B representing the same data in a logarithmic scale). This resulted in a strong linear association of R2 0.9861.



Figure 2. The deviation of chimerism percentages of individual measurements compared with the mean percentage and plotted against this mean percentage.

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Figure 3. The relative error plotted for each measurement against the mean percentage.

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