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Standardization and validation of PCR-fragment analysis based assay for detection of mixed donor chimerism following bone marrow transplant

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Abstract

Haematopoietic Stem cell transplantation (HSCT) is the treatment of choice for number of haematological disorders like Beta Thalassemia and certain type of leukemias, which is accomplished by either peripheral blood stem cell transplantation or most commonly by bone marrow transplantation (BMT). Although a promising therapy, disease remission or graft failure has been a major concern following transplant. PCR - fragment analysis based detection using short tandem repeat (STR) markers has been helpful in accurately measuring the percentage of healthy donor cells in the recipient's blood and can be instrumental in early diagnosis of disease relapse as well as graft versus host disease (GVHD). The aim of the present study was to standardize and validate the assay for detection of mixed donor chimerism following BMT. Sample collection: For standardization of the assay, blood samples were collected from four healthy volunteers and artificial chimeric samples with different grades of donor cell population (ranging from 1%-90% donor cells) were generated. For validation, blood samples from haploidentical donors and recipients collected pre-BMT were used for STR baseline analysis. For screening of mixed donor chimerism, blood samples from two patients for 6 intervals following autologous BMT were used. Baseline screening for informative STR markers was done for all the samples by PCR amplification for 9 STR loci viz. D3S3045, D4S2366, D12S1064, D16S539, D17S1290, SE33, FGA, D20S481 and AME, along with GAPDH as an internal control. The products were subjected to capillary electrophoresis using 3500 Genetic Analyser and the data were analysed using GeneMapper software. Further, PCR - fragment analysis was carried out for specific informative markers across all the artificial chimeric samples as well as post-BMT samples. For validation, the data was compared with another referral laboratory for inter laboratory comparison. For standardization, results for chimeric samples from healthy volunteers showed 99% accuracy for detection of expected donor chimerism values. For validation, results of inter-laboratory comparison using post-BMT samples from patients were in concordance (%CV≤5%). Accurate estimation of donor status in the post-BMT samples could be achieved upto 1% of donor cells.

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