ASSESSMENT OF OPERATOR VARIATION IN FLOW CYTOMETRY MEASUREMENTS USING GAUGE REPEATABILITY & REPRODUCIBILITY TECHNIQUES

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An existing large scale analysis of variation of starting materials for Hematopoetic Stem Cell Therapies has highlighted the potential for deviations of practice in operator use of flow cytometers at single centre clinical facilities. This has potentially led to a variation of Quality Control data within cell therapy bioprocessing at distinct stages such as sample preparation, cytometer setup, data processing and results interpretation. A quantitative understanding of operator impact on results would significantly contribute to meeting and defining manufacturing quality control standards, as well as progressing regulatory guidance for measurement uncertainty when interpretation of results is required to sign off material for testing and release.

Various efforts are being pursued to minimise this variation and the consequential impact on reported flow cytometry data. External Quality Assessment Schemes focus on inter-laboratory variation of results when a standard sample and uniform operator training are provided, whilst EuroFlow protocols provide a standard analysis template to use with local samples. Furthermore, automated software is abundant for flow cytometry, but is often met with scepticism among operators. Little has been published on intra-laboratory variation for flow cytometry, which is the basis of this developing research, providing a bottomup approach to quantification.

Gauge Repeatability & Reproducibility studies have been conducted with single site research, process, and quality control based participants from Loughborough University, GlaxoSmithKline and LGC. Participants analysed predefined Flow Cytometry data that encompassed a wide range of deviation, from simple histogram gating of overlapping peaks to full gating derivation of certain subsets of cells. Analysis of the results has shown large variation of output data and results between operators and contributory variation between operators with all levels of experience. However, application of even simple diagrammatical reference aids significantly reduced operator variation, providing supporting evidence for the introduction of more streamlined protocols to improve the confidence in, and the potential for enhanced precision of flow cytometer results.