Identification of NK-subsets: Hardware compensation of 4-colour tubes

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In this presentation the establishment of a multicolour flowcytometry protocol, conducted on a Beckman Coulter FC500, will be presented from a practical point of view. The protocol is aimed to characterise subsets of the circulating lymphocytes in peripheral blood - with special emphasis on the human Natural Killer (NK) cell system. In this 4-colour protocol we use the lymphocyte markers CD3, CD4, CD8, CD14, CD16, CD19, CD45, CD56 and CD57 as well as the activation markers CD69, CD25 and CD122. The fluorochromes used are FITC, PE, APC and PC7. Problems in relation to hardware and software compensation for fluorescence emission spectral overlap as well as the combination of flurochromes, gating strategies and the use of analyse software in relation to multicolour flowcytometry, will be discussed.

Examples of clinical and experimental applications of our multicolour flowcytometry protocol will be demonstrated stressing that multicolour flowcytometry is an important tool when changes in the NK cell system are investigated.