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Autor(en) des Beitrags:     Körber, Nina; Behrends, Uta; Hapfelmeier, Alexander; Protzer, Ulrike; Bauer, Tanja

Titel des Beitrags:         Validation of an IFN?/IL2 FluoroSpot assay for clinical trial monitoring.

Abstract:                  The FluoroSpot assay, an advancement of the ELISpot assay, enables simultaneous measurement of different analytes secreted at a single-cell level. This allows parallel detection of several cytokines secreted by immune cells upon antigen recognition. Easier standardization, higher sensitivity and reduced labour intensity render FluoroSpot assays an interesting alternative to flow-cytometry based assays for analysis of clinical samples. While the use of immunoassays to study immunological primary and secondary endpoints becomes increasingly attractive, assays used require pre-trial validation. Here we describe the assay validation (precision, specificity and linearity) of a FluoroSpot immunological endpoint assay detecting Interferon ? (IFN?) and Interleukin 2 (IL2) for use in clinical trial immune monitoring. We validated an IFN?/IL2 FluoroSpot assay to determine Epstein-Barr virus (EBV)-specific cellular immune responses (IFN?, IL2 and double positive IFN? + IL2 responses), using overlapping peptide pools corresponding to EBV-proteins BZLF1 and EBNA3A. Assay validation was performed using cryopreserved PBMC of 16 EBV-seropositive and 6 EBV-seronegative donors. Precision was assessed by (i) testing 16 donors using three replicates per assay (intra-assay precision/repeatability) (ii) using two plates in parallel (intermediate precision/plate-to-plate variability) and (iii) by performing the
assays on three different days (inter-assay precision/reproducibility). In addition, we determined specificity, linearity and quantification limits of the assay. Further we tested precision across the two assay systems, IFN?/IL2 FluoroSpot and the corresponding enzymatic single cytokine ELISpot. The validation revealed: (1) a high intra-assay precision (coefficient of variation (CV) 9.96, 8.85 and 13.05 %), intermediate precision (CV 6.48, 10.20 and 12.97 %) and reproducibility (CV 20.81 %, 12.75 % and 12.07 %) depending on the analyte and antigen used; (2) a specificity of 100 %; (3) a linearity with R (2) values from 0.93 to 0.99 depending on the analyte. The testing of the precision across the two assay systems, adduced a concordance correlation coefficient p c = 0.99 for IFN? responses and p c = 0.93 for IL2 responses, indicating a large agreement between both assay methods. The validated primary endpoint assay, an EBV peptide pool specific IFN?/IL2 FluoroSpot assay was found to be suitable for the detection of EBV-specific immune responses subject to the requirement of standardized assay procedure and data analysis.