Introduction

This work was designed to further validate the human Cytokine Panel V-PLEX™ Plus bioassay system launching Meso Scale Discovery (MSD®) using normal human serum (NHS) as sample matrix. The test kit allows for the simultaneous assay of 10 cytokines, including GM-CSF, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, GM-CSF, and TNF-β, using soluble biomarker panels. The results were then compared to the gold standard method validation parameters using NHS as matrix.

Methods

The assays were performed according to the manufacturer’s recommendations. NHS samples were screened for optimal minimum required dilution and endogenous levels of the listed cytokines. A pool of NHS was used to generate validation samples (VS) in the V-PLEX™ assay system. For the 10 assays, the VS contained at least 95% NHS. MSD® controls were reconstituted with MSD® Buffer (Diluent 43) and then serially diluted in NHS before diluting 1:4 in assay diluent. The VS were diluted 1:4 in buffer and then 1:4 in sample matrix. The VS were subjected to six accuracy and precision runs. The data presented identify methods that provided acceptable selectivity for all cytokines except IL-16 (LLOD ~ 2.7 pg/mL). Upper limits of quantitation (LLOQ) were subjected to six accuracy and precision runs. The data presented identify methods that did not provide acceptable selectivity for all cytokines except IL-16 (LLOQ ~ 2.7 pg/mL).

Results

Preparative and assay of CS, VS, and MSD® controls. The signal-decay calibration blend provided with the kit was reconstituted with MSD® Buffer-Diluent 43 and then serially diluted in Diluent 43 to obtain the desired concentrations for the standard curve. NHS samples were purchased from commercial suppliers and were subjected to 6 accuracy and precision runs. The data presented identify methods that did not provide acceptable selectivity for all cytokines except IL-16 (LLOD ~ 2.7 pg/mL). Upper limits of quantitation (LLOQ) were subjected to six accuracy and precision runs. The data presented identify methods that did not provide acceptable selectivity for all cytokines except IL-16 (LLOQ ~ 2.7 pg/mL).

Reproducibility of MSD® Controls

The calibration standard blend provided with the kit was reconstituted with MSD® Buffer-Diluent 43 and then serially diluted in MSD® Buffer-Diluent 43 to obtain the desired concentrations for the standard curve. NHS samples were purchased from commercial suppliers and were subjected to 6 accuracy and precision runs. The data presented identify methods that did not provide acceptable selectivity for all cytokines except IL-16 (LLOD ~ 2.7 pg/mL). Upper limits of quantitation (LLOQ) were subjected to six accuracy and precision runs. The data presented identify methods that did not provide acceptable selectivity for all cytokines except IL-16 (LLOQ ~ 2.7 pg/mL).

Conclusion

The results demonstrate that the R&D Systems assay kit can be adapted to fully validate status in NHS.