Cytokine Immunoassays with Sub-fg/mL Detection Limits

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Abstract

Serum and Plasma Samples

Typical serum concentrations of many cytokines (e.g., IL-2, IL-4) are well below 0.1 pg/mL and therefore are not measurable in most individuals. Our 20 normal individuals, and serum samples from 20 sepsis patients. These samples were also tested on an MSD V-PLEX panel. In addition, buffy coat stimulated with LPA, PMA, PMS, PWM, or concavalin A was tested.

Methods

A next-generation assay format based on MSD’s MULTI-ARRAY electrochemiluminescence technology was developed requiring only 25 µl of sample input. The assay requires no washes, no blocking, and no enzymatic reactions. Lower limits of detection (LOD) were quantitated for these assays requiring 0.025 µg/mL and 0.006 pg/mL for IL-2 and IL-6 respectively, compared to 0.1 µg/mL and 0.3 pg/mL respectively for ELISA assays. Typical intra-assay coefficients of variation (CVs) were 6% and 8% for IL-2 and IL-6 respectively. Inter-assay coefficients of variation (CVs) were less than 15% and 20% for IL-2 and IL-6 respectively. Median concentrations of normal samples tested. All samples were also measured with a commercial, antibody-based MSD assay. Both great concordance between the two formats were observed.

Results

Conclusion

Reproducibility

Approximately 80-100 serum or plasma samples were tested, including a set of matched serum, EDTA plasma, and heparin plasma samples from 20 normal individuals, and serum samples from 20 sepsis patients. These samples were also tested on an MSD V-PLEX panel. In addition, buffy coat stimulated with LPA, PMA, PMS, PWM, or concavalin A was tested.

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