Bio-Plex® suspension array system

tech note 5980

Performance Comparison of Multiplex Immunoassays Using Bio-Plex 200 and Bio-Plex 3D Systems

Vinita Gupta, Abraham Bautista Jr. and John Pocekay. Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA 94547 USA.

Introduction

Bio-Plex 3D system is a high-throughput next generation instrument based on xMAP technology. This system has a three-dimensional bead map that allows selection of up to 500 unique analytes for multiplexing within a single sample. Improvements in the system deliver read times twice as fast as the Bio-Plex 200 system. Other features such as 384-well plate compatibility, a robotics-compatible sample tray, robotics interfacing capabilities, and LIS-compatible software enable operation in an automated setting.

The new three-dimensional bead map (Figure 1) is a matrix of the emitted fluorescence of the three internal dyes on the x, y, and z axes. It is a three-dimensional graph with ellipsoid targets for each region. The original 1–100 bead map is the base of the 500-plex bead map with the new regions emerging from it as the third dye is added in increasing amounts.

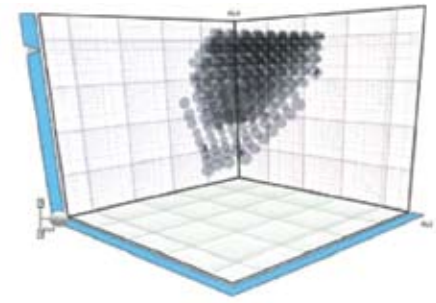

Fig. 1. Three-dimensional bead map for Bio-Plex 3D system.

In this study we have compared the performance of Bio-Plex Pro™ human 27-plex group I cytokine assays and Bio-Plex Pro mouse 23-plex group I cytokine assays analyzed on Bio-Plex 3D and Bio-Plex 200 systems.

Methods

Bio-Plex Pro human 27-plex group I cytokine assays were tested on the two systems using a 96-well plate. The standards, blanks, and serum samples were run in replicates of six as shown below in Figure 2. The replicates were pipetted on each half of the plate to be read on two separate platforms. Each half was transferred to two new plates prior to data acquisition. Enhanced PMT was set on the Bio-Plex 3D system whereas low PMT was set on the Bio-Plex 200 system for data acquisition. Bio-Plex Pro II wash station with magnetic plate carrier was used for washing the plates.

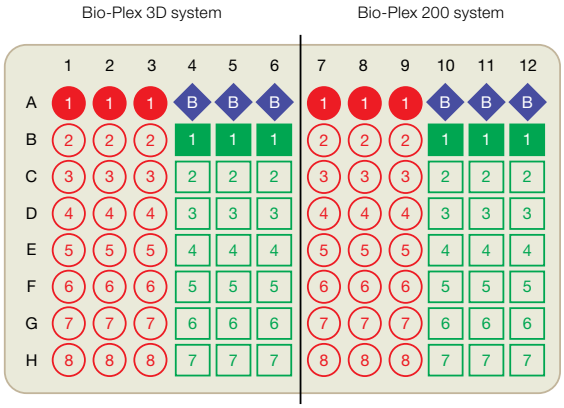

Fig. 2. Plate format for assay performance comparison.

The results from the two instruments were analyzed using Bio-Plex Manager™ software version 6.0 to keep the analysis method between the two instruments consistent. The performance of the assays was compared based on sensitivity, assay range, precision, accuracy, and sample concentrations.

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Results

Limit of Detection (LOD)

LOD is defined as the concentration corresponding to the minimum median fluorescence intensity (MFI) value that can be reliably differentiated from background and is two standard deviations from the appropriate blank values, which translates to 99% certainty. The LOD of human 27-plex assays, which is the mean value from three independent assays, demonstrates that the sensitivity of the majority of analytes is comparable. All the values were <8 pg/ml (Figure 3).

Background Signal, Dynamic Range, and Standard Recovery

Although the Bio-Plex 3D system can record data across a larger dynamic range than the Bio-Plex 200 system, the assays tend to saturate at the same concentration on both instruments. Additionally, the gain in MFI with the Bio-Plex 3D instrument is proportional for all points of the standard curve and the background (Figure 4). Therefore, the standard curve recovery, which reflects the accuracy of the assay was comparable between the two instruments.

Figure 5 depicts the representative standard curves of human 27-plex assays from data obtained using the two instruments.

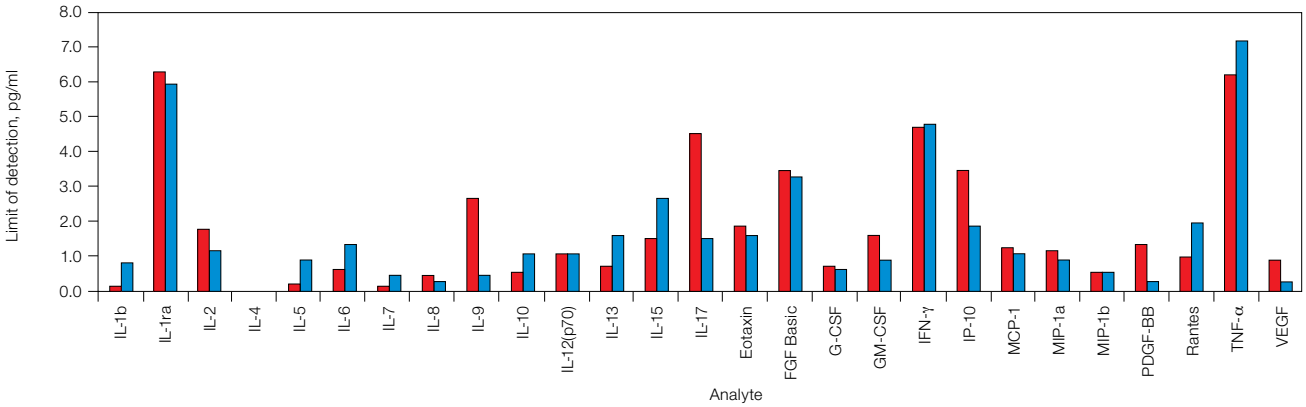

Fig. 3. LOD of Bio-Plex Pro human 27-plex group I cytokine assays using Bio-Plex 200 and Bio-Plex 3D systems. ■, Bio-Plex 200 system; ■, Bio-Plex 3D system.

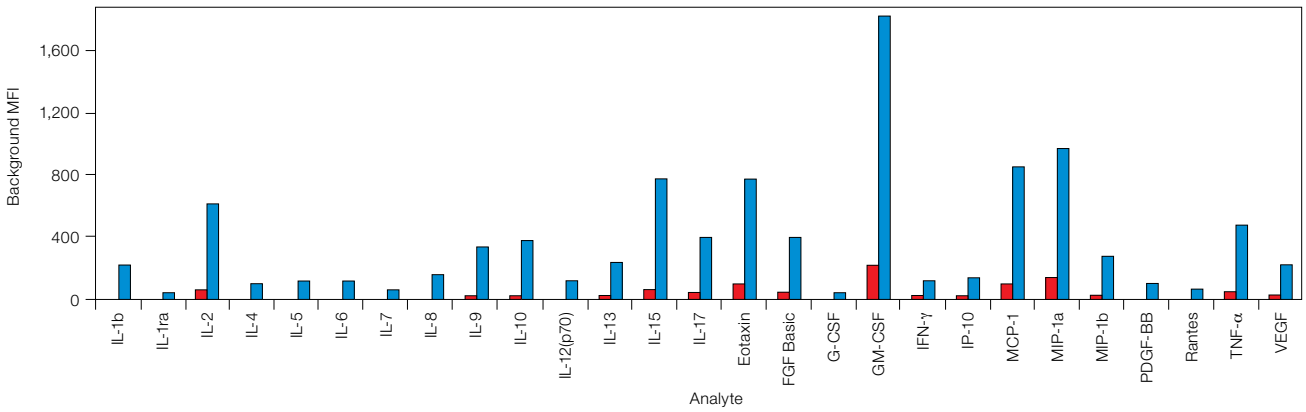

Fig. 4. Background MFI using the two instrument platforms. ■, Bio-Plex 200 system; ■, Bio-Plex 3D system.

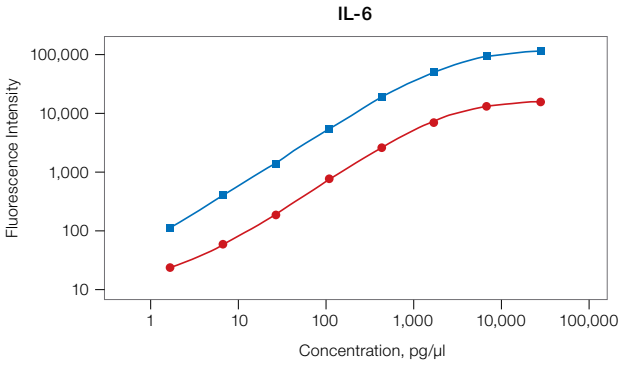

Fig. 5. Dynamic range of the curve obtained with the two instruments. ●, Bio-Plex 200 system; ■, Bio-Plex 3D system.

Precision

The inter-assay precision, calculated using observed concentration of standards from three independent experiments was similar for all targets (Figure 6). The %CV for all the analytes was <12%.

Working Assay Range

The working assay range is defined as the standard curve range where the analytes meet the recovery of 70–130% and intra-assay precision of 20%. As shown in Figures 7, the assay ranges for most of the analytes were unaffected by the wider dynamic range of the Bio-Plex 3D instrument. Lower limits of quantitation (LLOQ) for all the analytes were <7 pg/ml. The higher LLOQ for IL-17, IFN-γ and IP-10 on the Bio-Plex 200 system was due to assay variability on one out of three independent assays. The upper limit of quantitation (ULOQ) for TNF-α was higher on the Bio-Plex 3D system due to assay variability observed at the saturation point of the curve with the Bio-Plex 200 system.

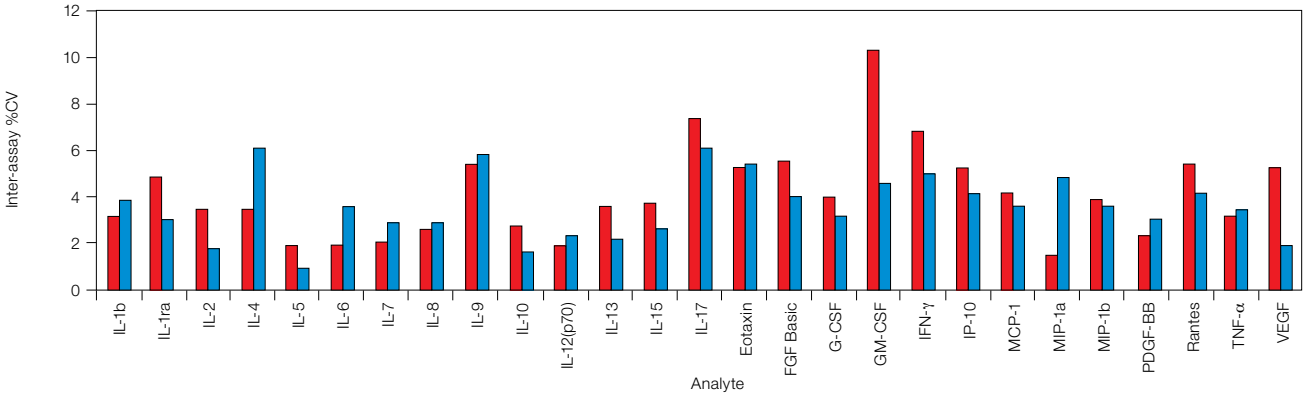

Fig. 6. Inter-assay %CV of observed concentration of standards. ■, Bio-Plex 200 system; ■, Bio-Plex 3D system.

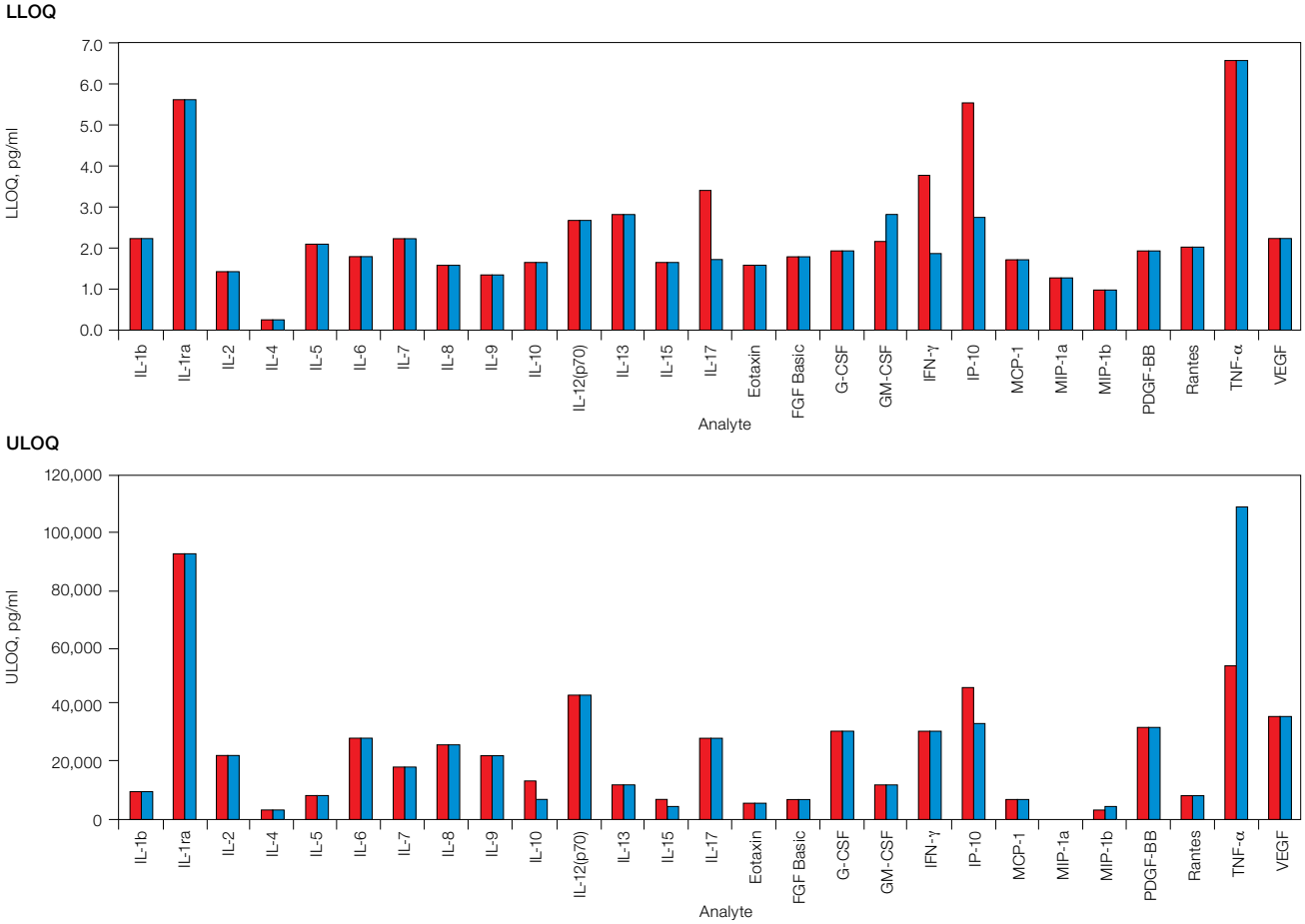

Fig. 7. LLOQ and ULOQ obtained using the two instrument platforms. ■, Bio-Plex 200 system; ■, Bio-Plex 3D system.

Sample Concentrations

Six serum samples were evaluated for concentration measurements on the two systems. Figure 8 shows the standard curve comparison when the same assay was run on Bio-Plex 200 and Bio-Plex 3D systems using Bio-Plex Manager 6.0 software. The standard curves had similar shapes and slopes. The samples fell within the same region of the standard curves. These curves are representative assays of Bio-Plex Pro human 27-plex group I cytokines tested on both systems.

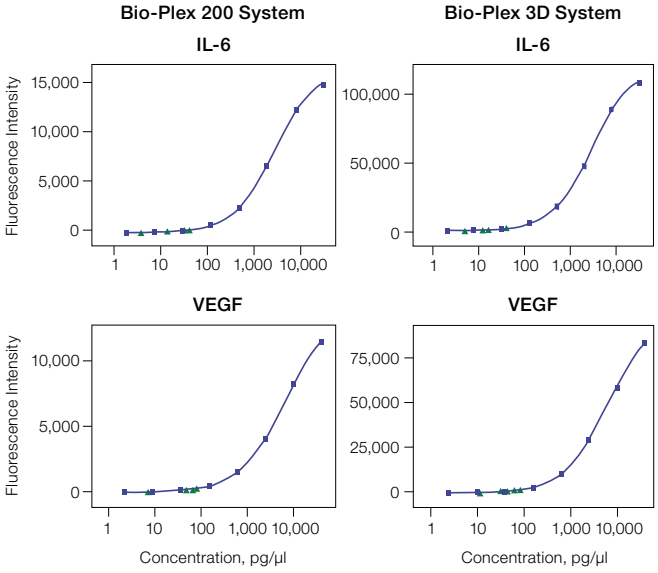

Fig. 8. Range of standards where sample concentrations fall.

Figure 9 depicts the concentrations of samples determined using the two systems. The concentration values were the same for all the samples tested with Bio-Plex Pro human 27-plex group I cytokine assays.

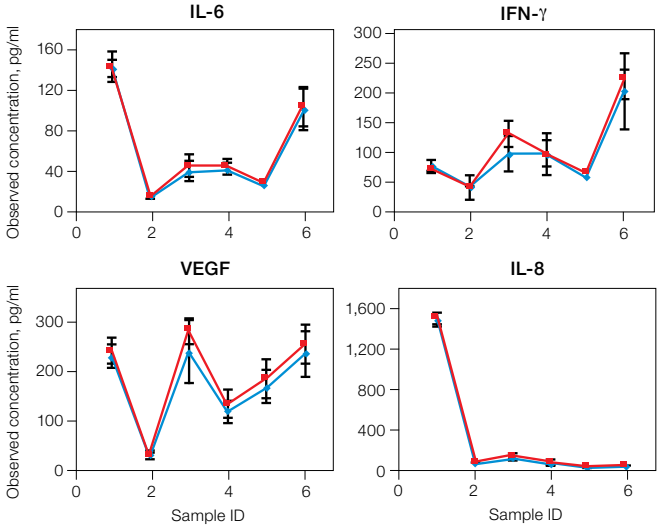

Fig. 9. Sample concentration comparison for Bio-Plex Pro human 27-plex group I cytokine assays. Concentrations of IL6, IFN-γ, VEGF and IL-8 were measured in six different samples using the two instruments. Error bars represent standard deviations from three independent assays. ■, Bio-Plex 200 system; ◆, Bio-Plex 3D system.

Data Acquisition Time

The data acquisition time for a 96-well plate on the Bio-Plex 3D system was 20 min whereas the time on the Bio-Plex 200 system was 40 min. The dual syringe capability leads to faster reading times on Bio-Plex 3D system and higher throughput results.

Conclusions

- Based on assay parameters evaluated, the new Bio-Plex 3D system performs in a similar manner as Bio-Plex 200 system
- Although Bio-Plex 3D system can record data across a wider dynamic range, the working assay range for all the analytes was similar
- Bio-Plex 3D system, due to dual sampling channels, has faster read times leading to higher throughput

Information in this tech note was current as of the date of writing (2010) and not necessarily the date this version (rev A, 2010) was published.

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