

Introduction

A critical element with the use of Luminex® microspheres is the adequate removal of unbound materials from the beads during the assay procedure. With increased throughput demands, the use of automated washers such as the ELx50™ Microplate Strip Washer and the ELx405™ Microplate Washer has been employed. In order for an adequate wash process to take place, while at the same time providing for sufficient bead recovery to allow measurement determinations to be timely and statistically valid, the configuration of the washer settings is critical. The automated washing of polystyrene MicroPlex® Microspheres with filter plates requires coordination between fluid levels and evacuation time to avoid impregnating the beads into the filter matrix. Automated washing of MagPlex® Microspheres, which can be immobilized with magnets, is much less problematic than vacuum aspiration provided that the proper washer settings are employed. The spatial relationship between the localization of the magnetic beads by the magnet and the aspiration tubes is paramount for bead recovery. Here we describe the washer parameters associated with low residual volumes, good bead recovery and optimal washing of microspheres. Human Cytokine Multiplex Assay kits from Bio-Rad and Invitrogen were used to optimize the ELx50 and ELx405 washers.

Vacuum Wash Workflow

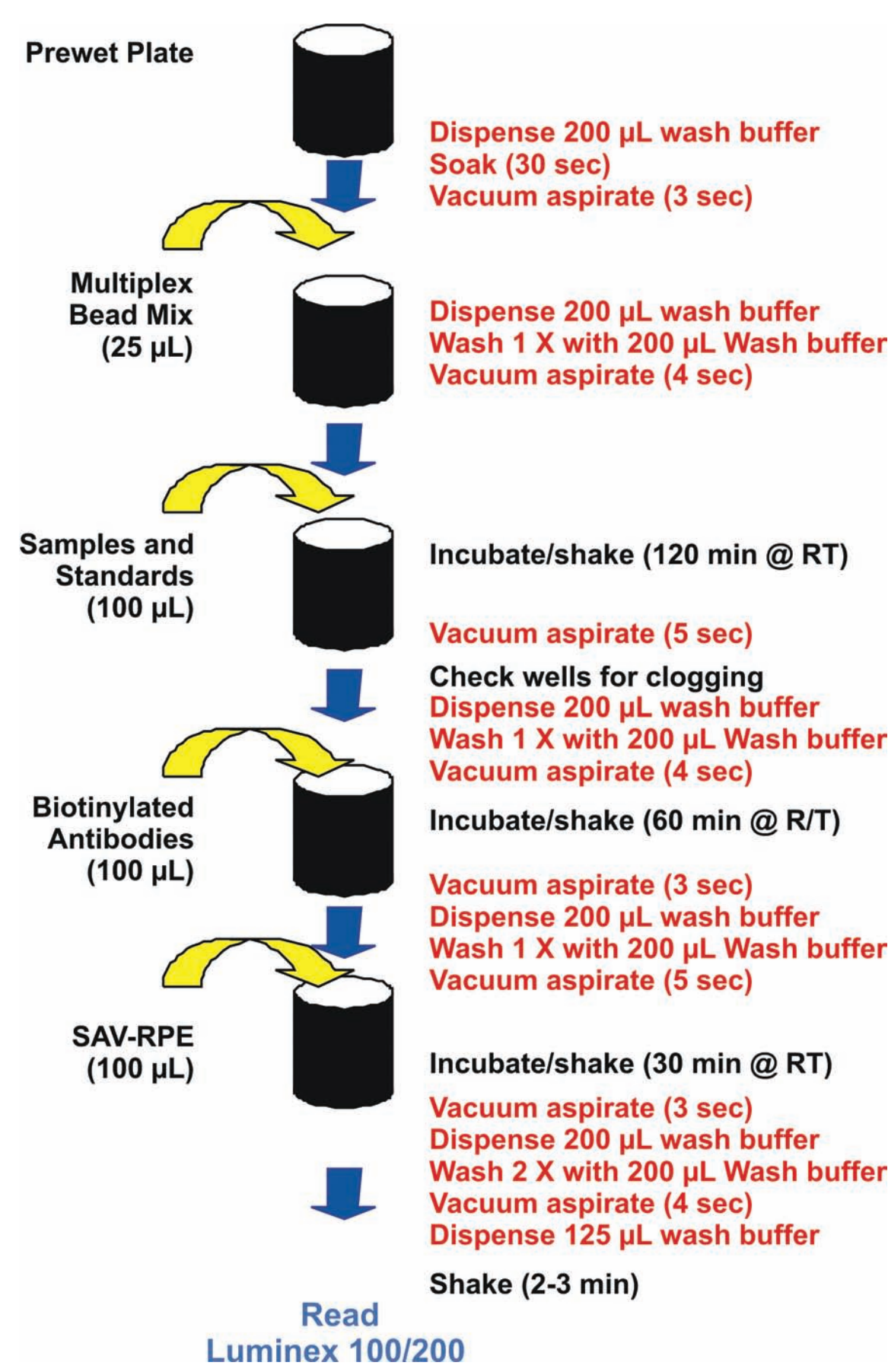


Figure 1 – Invitrogen Human Cytokine Assay vacuum wash protocol.

The filter plate was pre-wet using a one-cycle wash with a 30 second soak prior to aspiration. After wetting, 25 µL of the assay kit bead mix was added, and the beads were washed one time using assay wash buffer. Working multiplex standards were generated by serial dilution of the reconstituted human cytokine standard. These standards contained 10 different analytes. After reconstitution, 100 µL each of standards and samples were pipetted into bead containing wells of the assay microplate. The reaction was allowed to incubate for 120 minutes at room temperature (RT) with agitation on a plate shaker. After incubation, the plate was vacuum aspirated, and then manually checked for incomplete aspiration, commonly caused by clogged wells in the filtration plate, followed by two dispense and vacuum aspirations. After washing, 100 µL of detection or secondary antibody reagent was added and allowed to incubate for 60 minutes at RT with agitation. The beads were again washed three times followed by the addition of 100 µL of SAPE reagent. After a 30-minute incubation with agitation to allow for reporter tag binding to occur, the plate was again washed as described in the washing instructions. The samples and standards were then resuspended in 125 µL of assay buffer. Samples were then read on a Luminex 100 Reader with xPONENT® 3.1 Software using the parameters outlined in the assay kit instructions (Figure 1).

ELx50 Vacuum Wash Bead Recovery

	Manual	ELx50
Acquisition Time (sec)	14.8	18.5
Total Events (beads)	1322	1312
Acquisition Rate (beads/sec)	89.2	71.0

Table 1 – Comparison of manual and automated method acquisition time.

Bead recovery was also assessed by observing the acquisition time of separate wells in the 10-plex assay as part of the data assay analysis. The Luminex Reader was configured to count at least 100 beads of each type.

ELx50 Microplate Strip Washer



Figure 2 – ELx50 Microplate Strip Washer. BioTek's ELx50 is Luminex xMAP® approved.

Polystyrene Bead Vacuum Wash

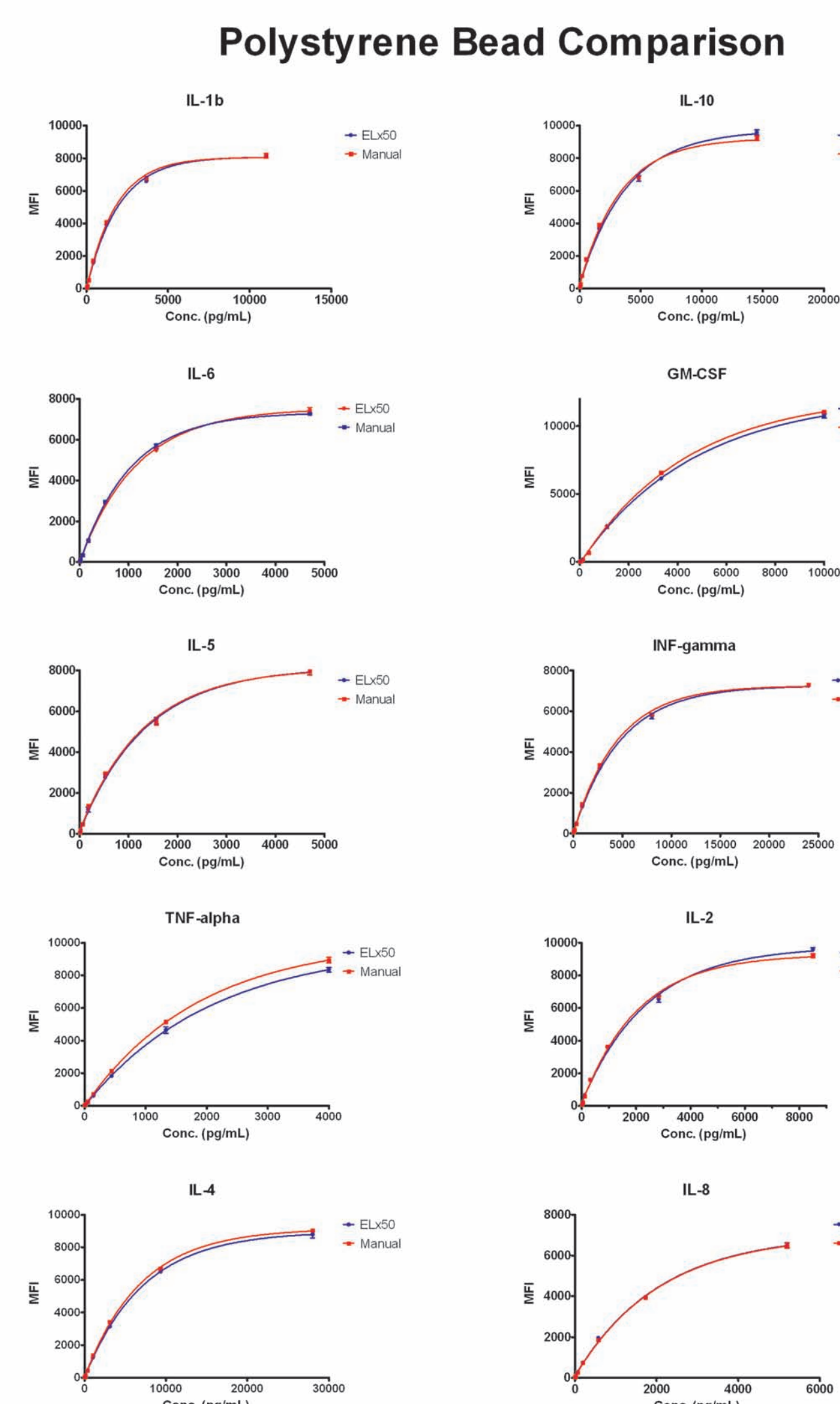


Figure 3 – Comparison of ELx50 and manual vacuum washed cytokine standard curves. Individual calibration curves from a 10-plex Invitrogen Cytokine Assay were plotted. Data points represent the mean and standard deviation of 4 data points.

Vacuum Wash Technology Comparison

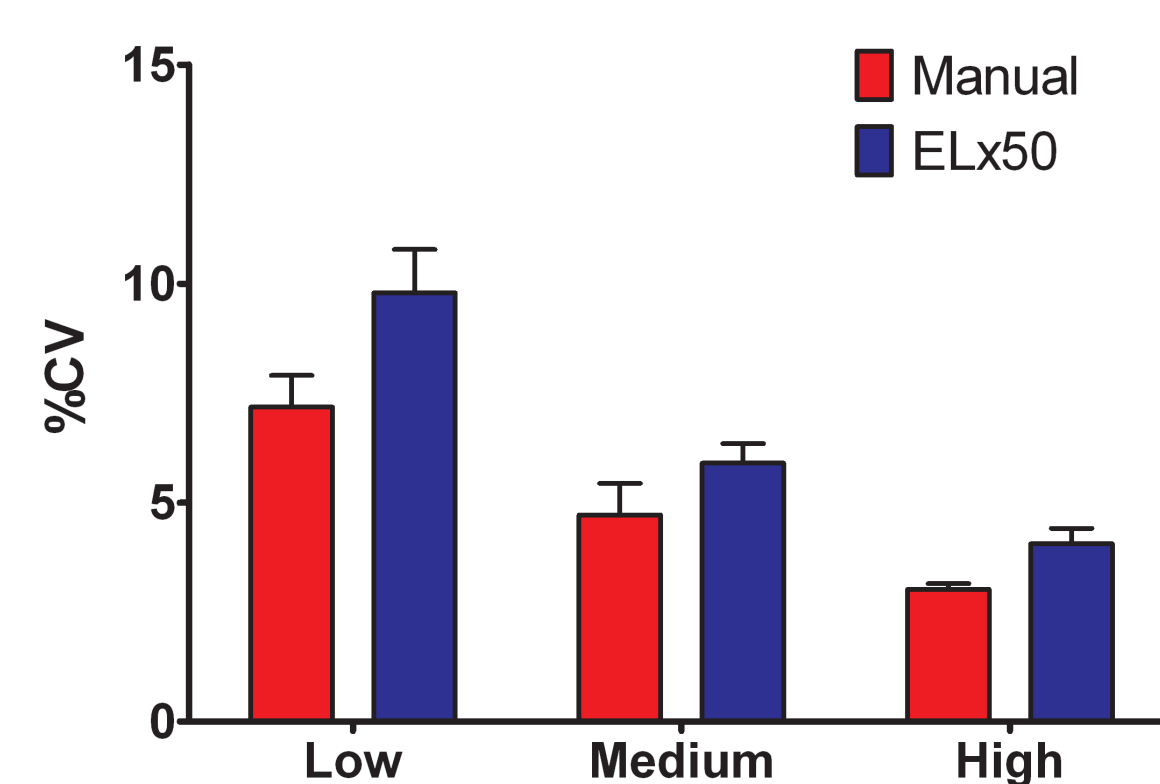


Figure 4 – Comparison of manual and automated vacuum wash methods intra-assay variability. The average %CV of ten different analytes measured at three different concentration ranges (Low, Med, High) was plotted for manual and ELx50 vacuum wash procedures. Data represent 16 determinations at each concentration range on the same assay plate.

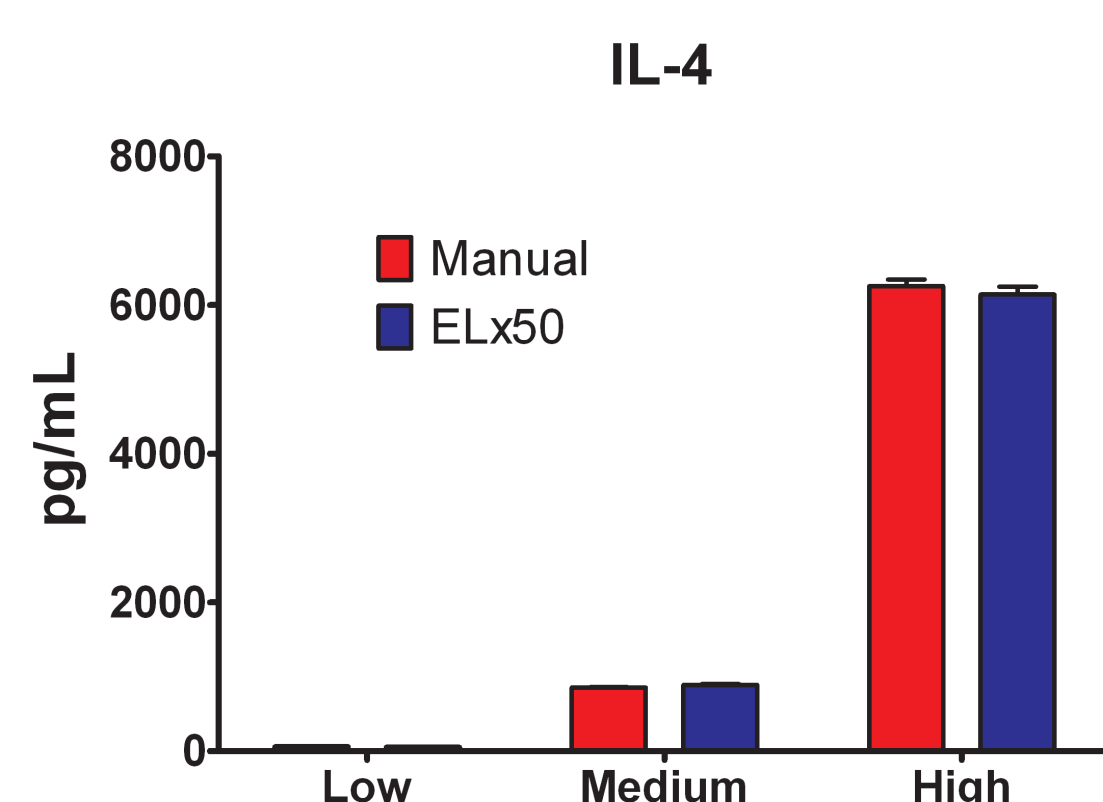


Figure 5 – Comparison of determined IL-4 concentrations using manual and ELx50 vacuum wash. Concentrations were calculated by interpolating MFI values from a standard curve. Data represent the mean and standard deviation of 16 data points.

Magnetic Wash Workflow

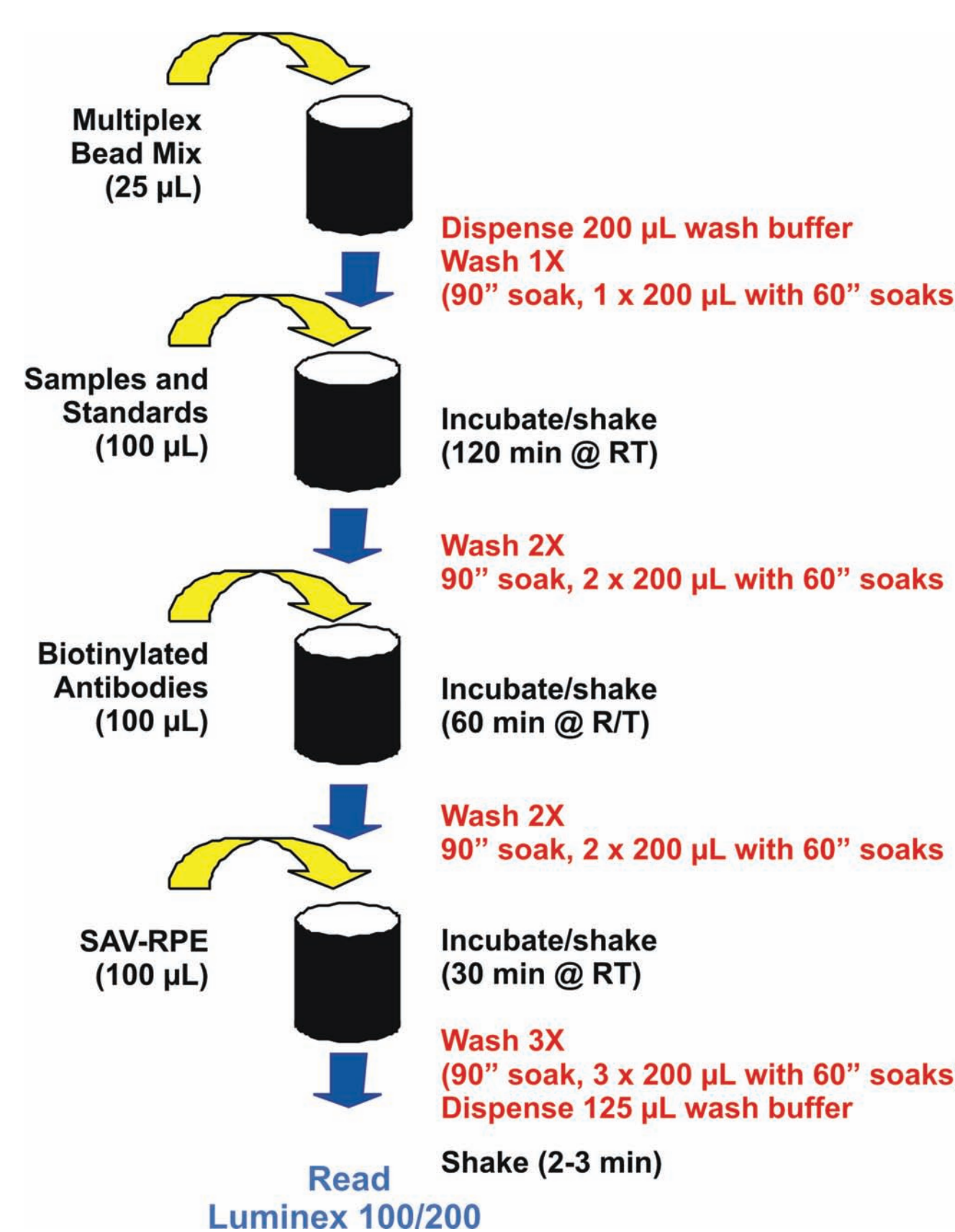


Figure 6 – Invitrogen Human Cytokine Assay magnetic wash protocol.

ELx50 Magnetic Wash Bead Recovery

	Manual	ELx50
Acquisition Time (sec)	10.88	10.97
Total Events (beads)	1426	1380
Acquisition Rate (beads/sec)	131.0	125.8

Table 2 – Comparison of manual and automated method acquisition times. Bead recovery was assessed by observing the acquisition time of separate wells in the 10-plex assay as part of the data assay analysis. The Luminex Reader was configured to count at least 100 beads of each type.

Magnetic Wash Technology Comparison

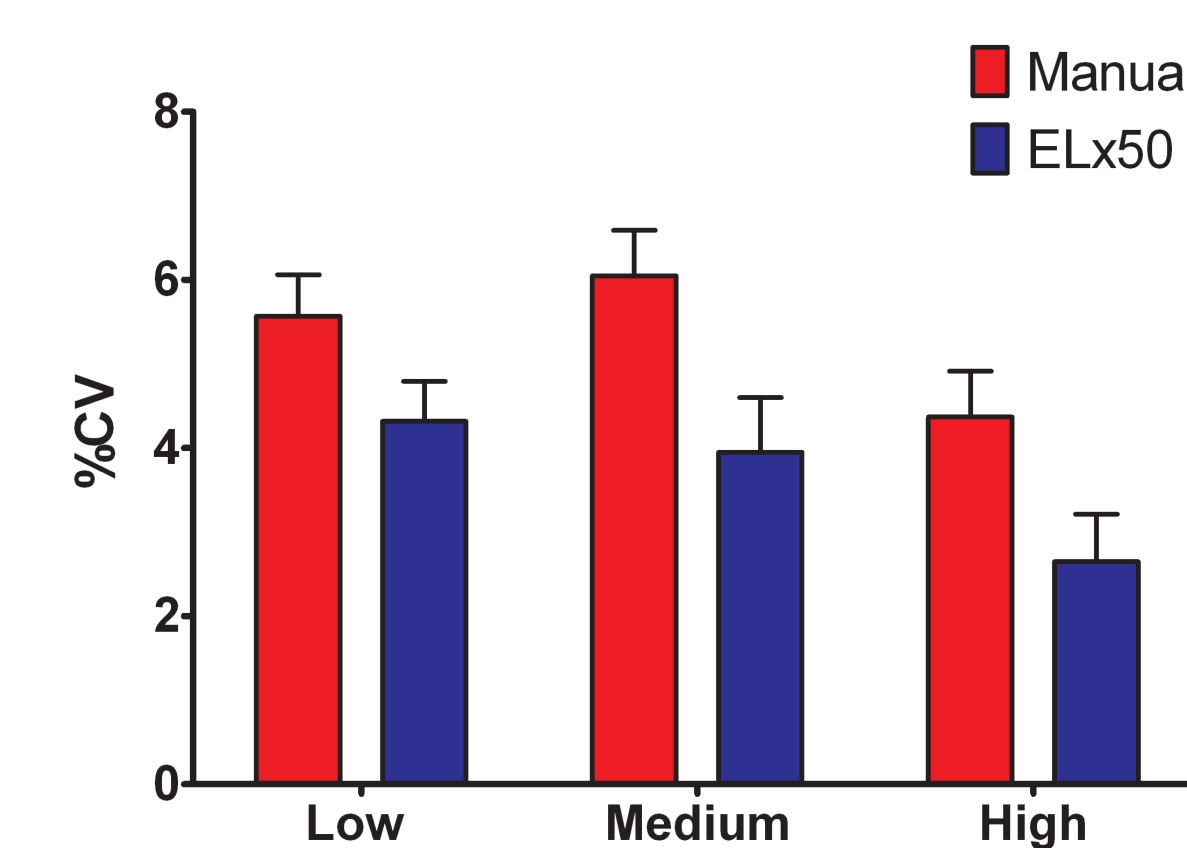


Figure 7 – Comparison of manual and automated magnetic wash methods intra-assay variability. The average %CV of ten different analytes measured at three different concentration ranges (Low, Med, High) was plotted for a manual and ELx50 magnetic wash procedures. Data represent 16 determinations at each concentration range on the same assay plate.

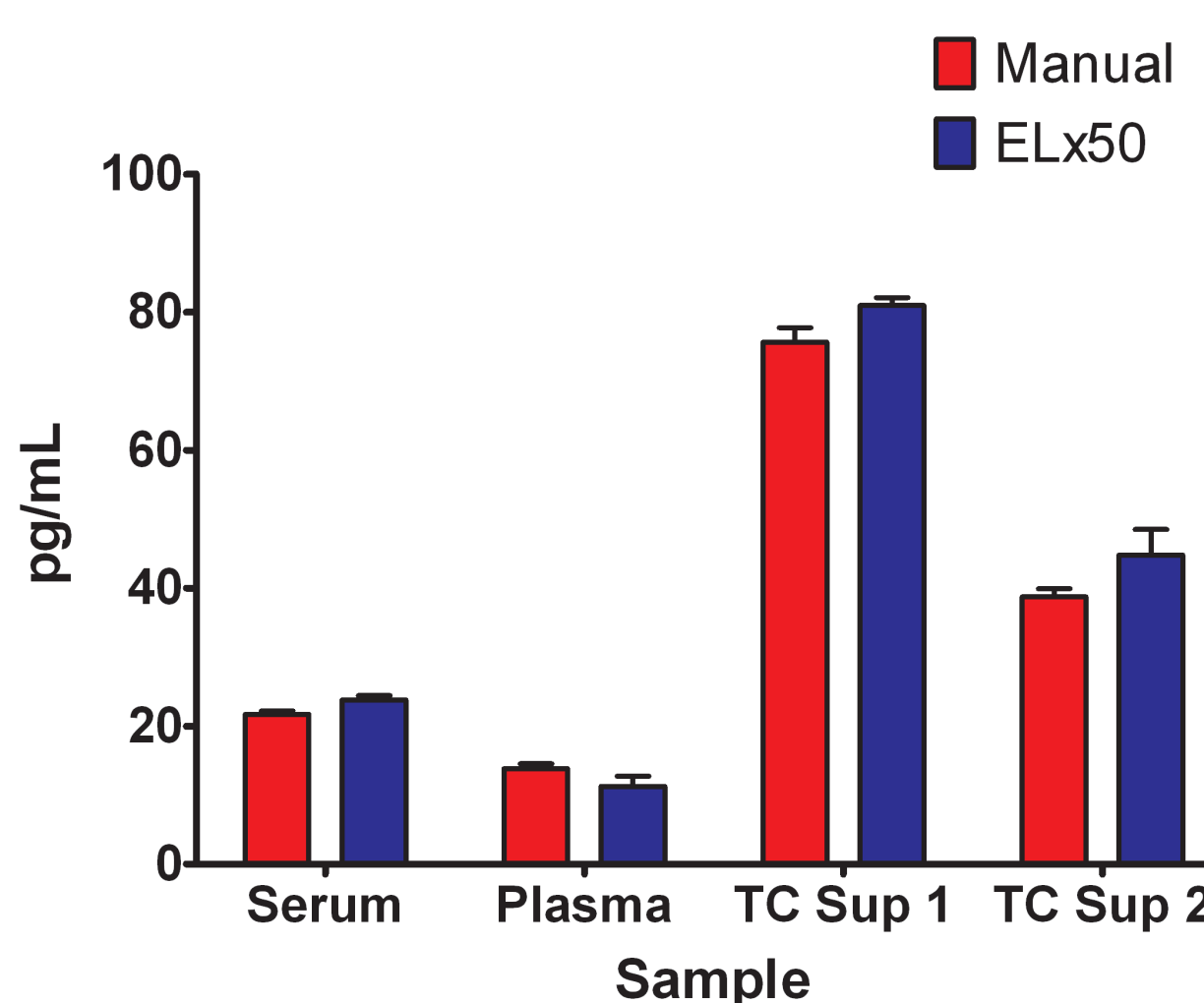


Figure 8 – Comparison of determined concentrations of natural samples. Lipid cleared samples of human pooled serum and plasma along with tissue culture media supernatant from cultured HEK293 cells were assayed for human IL-8 using magnetic beads. Washes were performed using an ELx50 as well as run manually. Data represent the mean of 4 determinations.

ELx405 Microplate Washer



Figure 9 – ELx405 Microplate Washer. BioTek's ELx405 is Luminex xMAP® approved.

ELx405 Magnetic Wash Bead Recovery

	Exp 1	Exp2
Acquisition Time (sec)	7.22	6.19
Total Events (beads)	498.2	500.88
Acquisition Rate (beads/sec)	69	81

Table 3 – Comparison of acquisition times from two separate assay experiments.

Bead recovery was assessed by observing the acquisition time of separate wells in the 8-plex assay as part of the data assay analysis. The Luminex Reader was configured to count at least 50 beads of each type. As demonstrated in Table 3, the acquisition time from two separate assay experiments, each with 27 different wells of the 8-plex assay, averaged approximately 7 seconds. The two separate runs exhibited very similar times, indicating that washer performance is consistent.

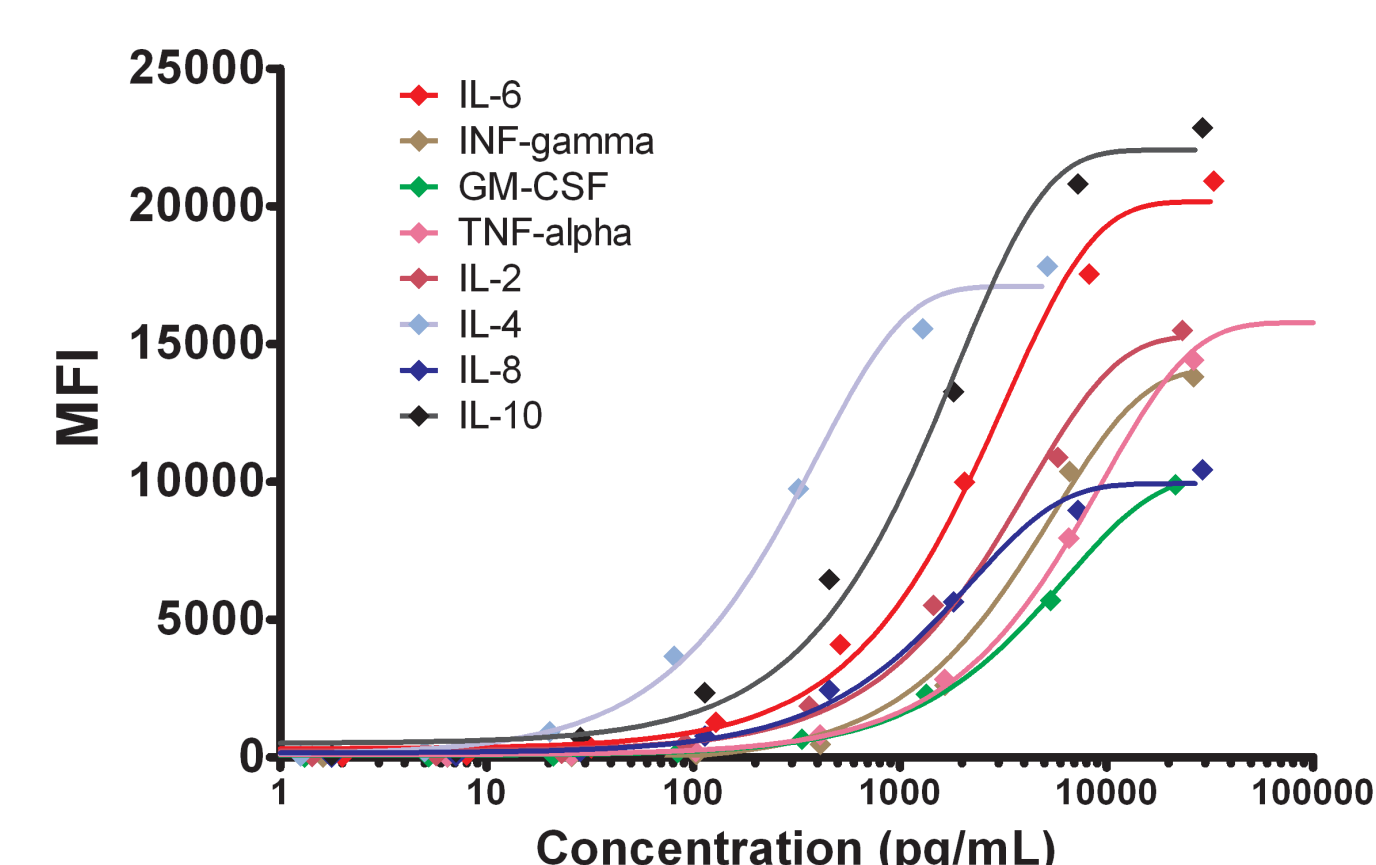


Figure 10 – Standard curves for the Bio-Rad Bio-Plex® 8-Plex human cytokine panel using ELx405 Microplate Washer.

Using known concentrations of analyte, a series of standard curves were generated for each cytokine by plotting the median fluorescent intensity (MFI) signal against concentration. These standard curves can then be interpolated to determine the concentrations of unknown samples. As with ELISA reactions, in order to obtain useable results, efficient washing to remove nonspecific antibody binding is critical. As shown in Figure 4, using the ELx405 Microplate Washer to wash magnetic bead-based multiplex assays in a 96-well microplate format results in very reliable data. These standard curves can be used to calculate unknown sample concentrations with a high degree of confidence.

Repeatability

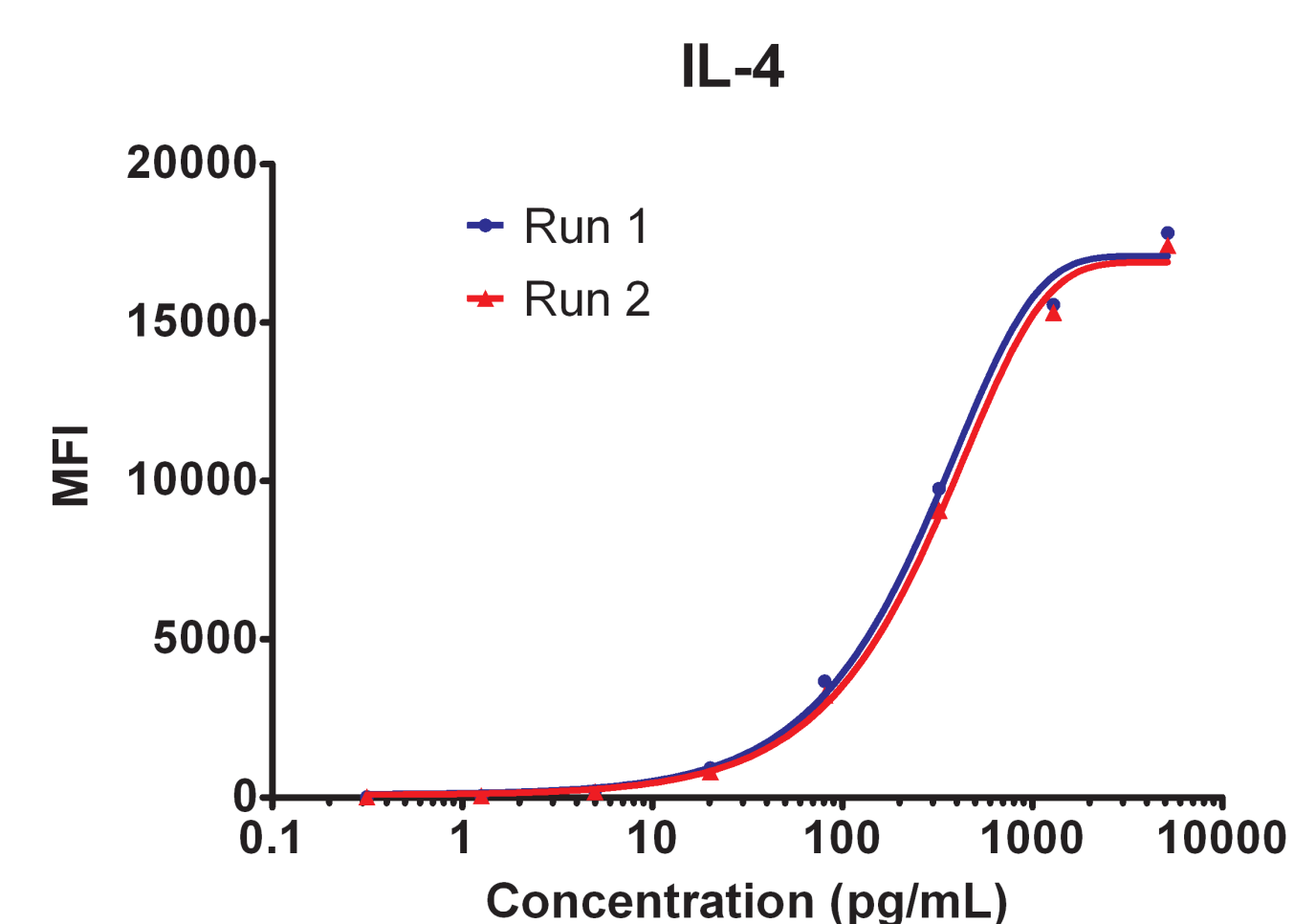


Figure 11 – Comparison of IL-4 standard curve from multiple runs.

Consistency of the assay results was tested by running the assay on subsequent days. As shown in Figure 11, day-to-day consistency for the assay is quite good. The median fluorescence intensity (MFI) of calibrations curves for IL-4 experiments run on two different days is virtually identical. Similar results for the other analytes of the 8-plex assay were also observed.

Conclusions

1. ELx50 Microplate Strip Washer provides equivalent performance relative to manual methods using polystyrene MicroPlex beads.
 - Improved Ease of Use
 - Equivalent Standard Curves
 - Equivalent Sample Concentrations
 - Equivalent Reproducibility
2. ELx50 Microplate Strip Washer provides comparable performance to the ELx405 Microplate Washer.
3. ELx405 Microplate Washer and ELx50 Microplate Strip Washer provide improved performance relative to manual methods using magnetic MagPlex beads:
 - Automated Sample Processor for xMAP Based Assays
 - More Rapid Sample Processing
 - Greater Wash Efficiency
 - Improved Ease of Use
 - Greater Assay Precision