

Automated Washing of Cancer Biomarker Assays

Comparison of LX100™ and MAGPIX® Reader Results



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Abstract

Diagnostic biomarkers are a key element in cancer research. Recently attention is being placed on identifying soluble extracellular circulating biomarkers, which can provide information on the body response to cancer, as well as the relationship between a tumor cell and its environment. Because cancer is a series of different disease states, the study of individual biomarkers is usually inadequate to study the complex relationship between a tumor and its environment. While some biomarkers are tumor specific, such as PSA, others such as IL-8, are found in tumors of many different origins. Using a panel of known tumor biomarkers to characterize tumor cell lines of known lineage under different conditions provides a better understanding of the biology specific to different tumor types. The advent of multiplex assays using Luminex Bead technology has simplified the task of performing multiple assays on samples. Luminex xMAP technology provides the means to measure multiple analytes simultaneously from the same sample. Originally designed around polystyrene MicroPlex® beads which required vacuum aspiration for washing, the latest generation of MagPlex® beads use embedded ferrite particles to allow for the use of magnets to immobilize the microspheres during the wash steps. Both beads can be read using a flow cytometry based reader that interprets the bead type as well as quantitate the analyte. Recently, a new paradigm of xMAP Reader has been provided by Luminex which does not utilize flow cytometry principles for detection. The MAGPIX® Reader System has been developed by Luminex exclusively for use with MagPlex microspheres. With both reader technologies, distinct internally color-coded magnetic microspheres coated with a specific antibody, capture and quantitate different analytes. Using traditional Luminex flow cytometry technology, microspheres were channeled to pass rapidly and individually through a laser beam which excites the internal dyes identifying the bead and analyte, while a second laser excites the reporter molecule, quantifying the analyte. The MAGPIX reader used in this study employs CCD fluorescent imaging to identify and quantitate the analyte. Both technologies require the same assay process prior to multiplex analyte determination by the reader. While we have established that the wash steps for MagPlex beads can be automated using an appropriately configured microplate washer using the LX100 reader, little data exists using the MAGPIX reader in conjunction with automated wash systems. Here we compare the results obtained with the MAGPIX reader to that of the LX100™ using the ELx50 to automate the wash steps of a EMD Millipore Cancer Biomarker 22-plex multiplex.

Assay Process

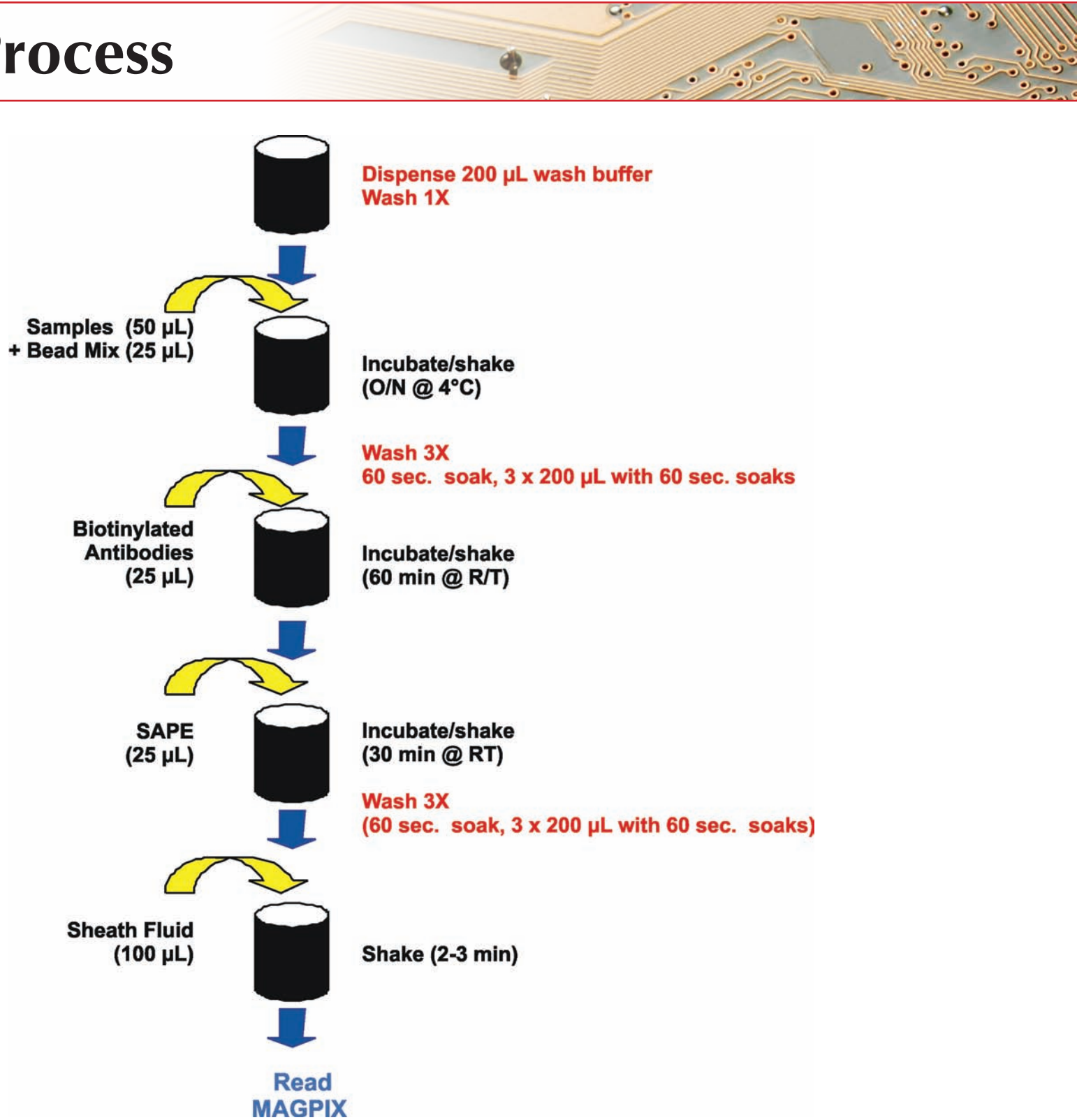


Figure 1 – EMD Millipore Cancer Biomarker Assay Process. Red text indicates automated processes carried out by the ELx50™ Microplate Strip Washer.

The MILLIPLEX assay was performed according to the kit instructions (Figure 1). Briefly, the assay plate was first washed once using the supplied assay wash buffer to remove any residue. Eight working multiplex standards were generated by serial dilution (1:3) of the reconstituted human cytokine standard. These standards contained 22 different analytes. After reconstitution, 50 µL each of standards and samples were pipetted into bead containing wells of the assay microplate. In parallel, the bead master mix was prepared by combining 150 µL of each individual bead suspension. 25 µL aliquots of the master mix were added to each well and the reactions were allowed to incubate overnight at 4°C with agitation on a plate shaker. The following day the plate was washed 3 times as described in the washing instructions. After washing, 25 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 25 µL of SAPE reagent. After 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 100 µL of sheath fluid. Samples were then read on either a Luminex MAGPIX or a LX100 reader with XPONENT software using the parameters outlined in the assay kit instructions.

Calibration Curves

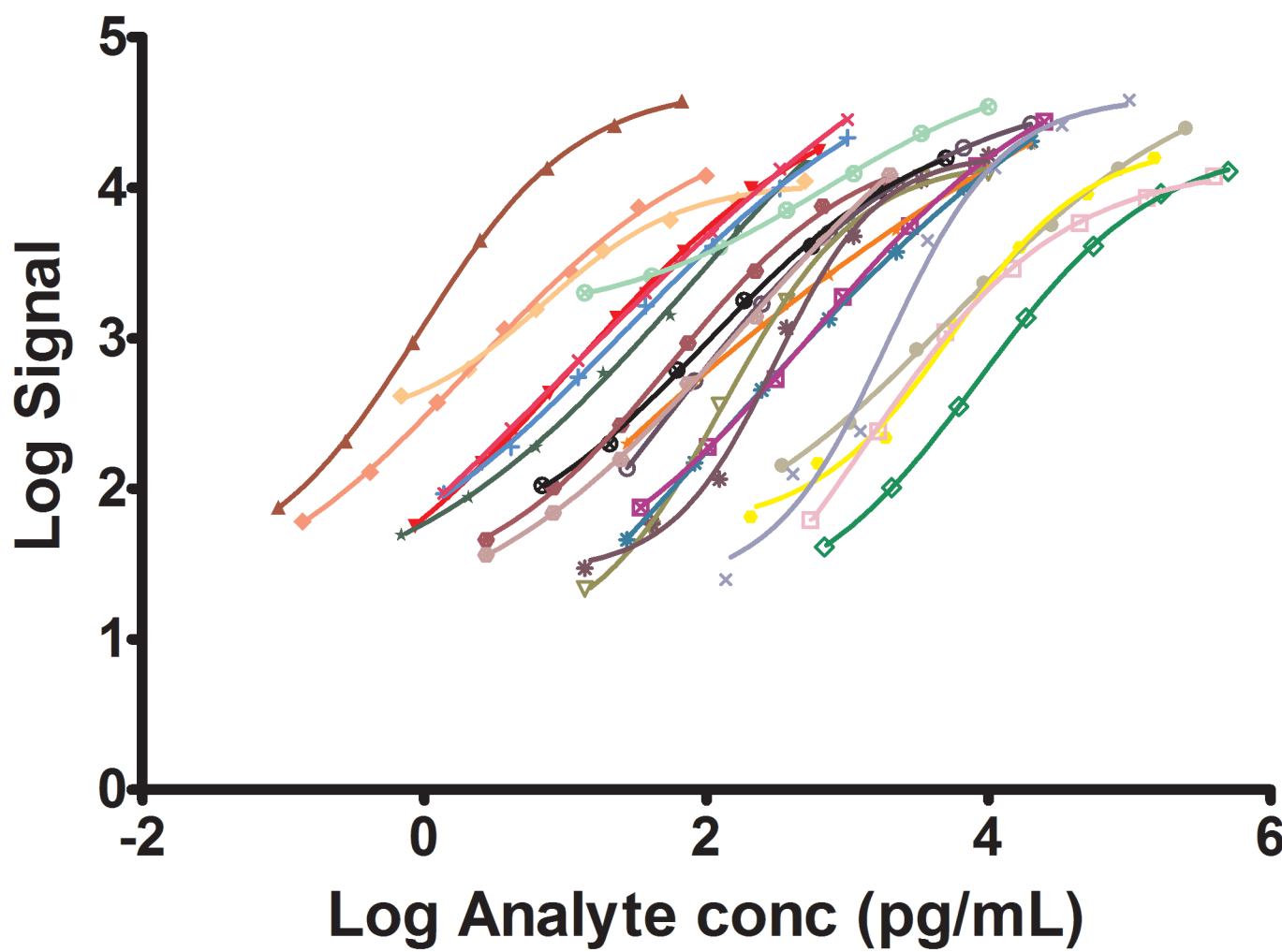


Figure 2 – Standard Curves for the EMD Millipore 22-Plex human Circulating Cancer Biomarkers using ELx50 Microplate Strip Washer.

Quality Control

Analyte	QC1		QC2	
	Expected Range	Result	Expected Range	Result
AFP	3319-6893	5035	16333-33921	28457
β-HCG	0.73-1.52	1.07	4.36-9.06	7.97
CA125	7.0-14.6	11.77	38-79	62.47
CA15-3	1.17-2.44	1.99	6.4-13.2	12.14
CA19-9	6.8-14.2	14.2	35-73	60.91
CEA	231-480	473	1204-2502	2308
CYFRA21-1	2206-4581	2665	11498-23881	18518
FGF2	123-255	143	596-1238	1045
HE4	7878-16361	10793	38636-80244	73437
HGF	224-465	254	1240-2574	1684
IL-6	4.56-9.48	9.29	26-54	46.9
IL-8	12-25	19.78	65-136	119
Leptin	1296-2691	1758	6399-13291	12430
MIF	330-685	647	1314-2729	2697
OPN	5592-11614	10907	28302-58782	56651
SCF	62-129	98.3	305-633	564.9
sFas	247-513	409.4	1340-2783	2497
TGF-α	24-50	35.5	131-273	205.2
TNF-α	12-26	22.9	67-140	117.6
Total PSA	125-261	255.5	650-1350	1196
TRAIL	26-54	45.4	136-283	219.9
VEGF	120-249	120.3	675-1402	899.8

Table 1 – Quality Control Analysis of human circulating biomarker 22-plex assay.

Calibration Curve Comparison

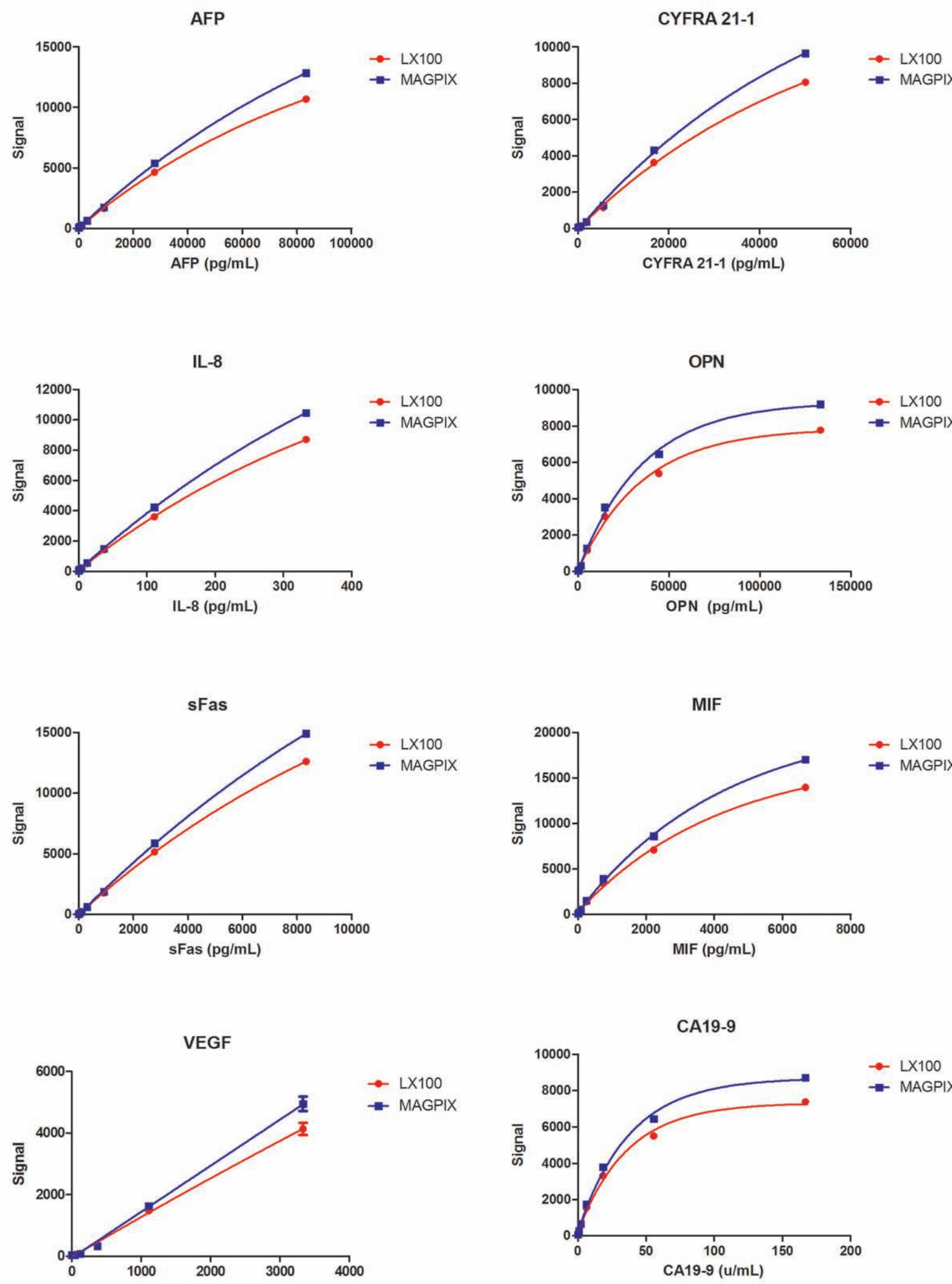


Figure 3 – Comparison of Select Calibration Curves. Calibration curves generated using either an LX100 or a MAGPIX reader from equivalent samples.

Cell Line Characterization

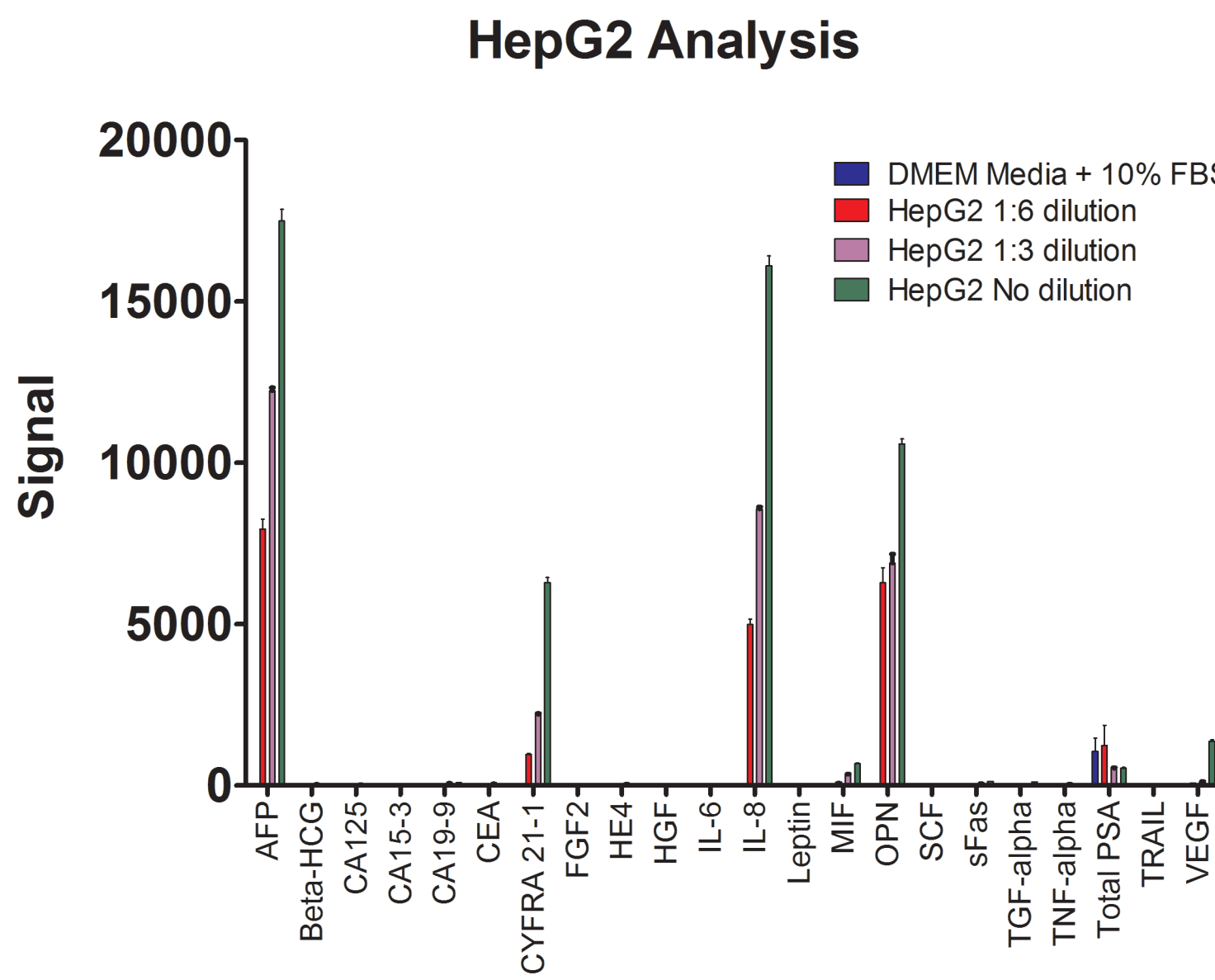


Figure 4 – HepG2 Cell line Biomarker characterization. Tissue culture cell conditioned media supernatant was obtained from HepG2 cultures. The conditioned media was diluted 1:3 and 1:6 with fresh media (DMEM + 10 % FBS) and aliquots (25 µL) were assayed in parallel with undiluted conditioned media. Data are the mean of 8 determinations.

ELx50 Washer



Figure 5 – ELx50 Microplate Strip Washer for bead washing.

Determined Results Comparison

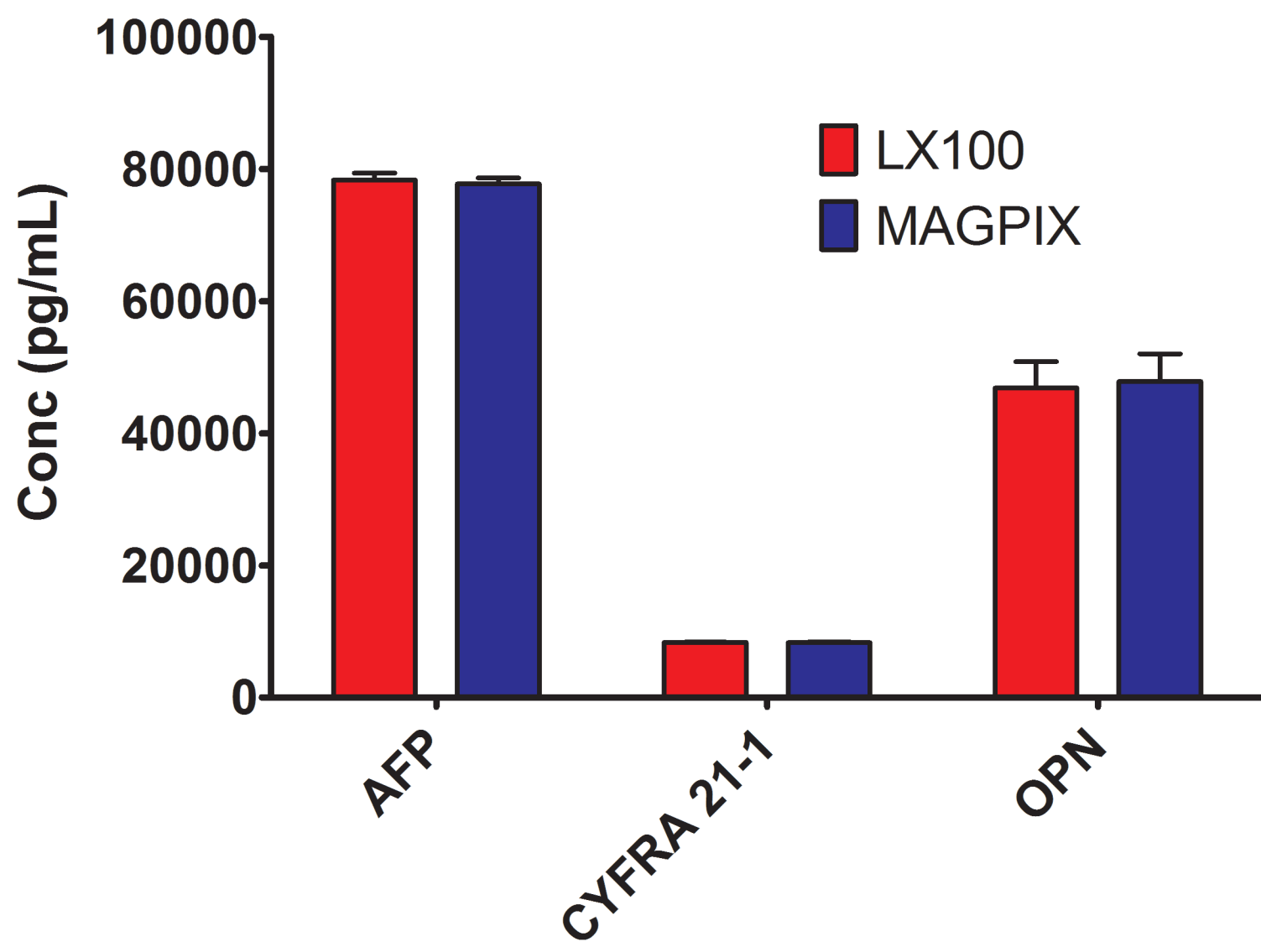


Figure 6 – Comparison of LX100 and MAGPIX Calculated Biomarker Concentrations. Multiplex reactions of HepG2 cell supernatant were assayed for the indicated cancer biomarkers along with standard curves in parallel using LX100 and MAGPIX readers. Determined concentrations from both readers are compared.

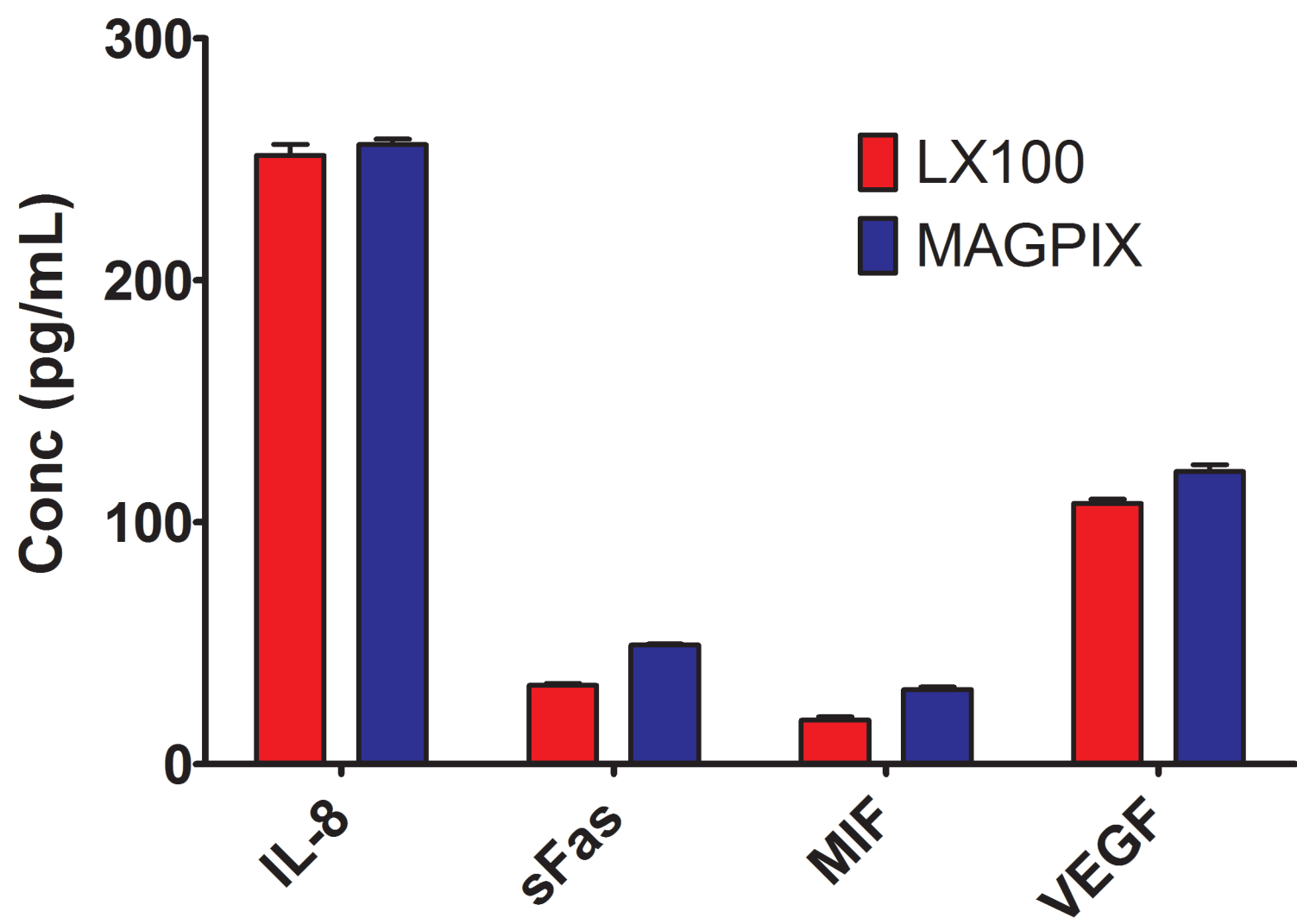


Figure 7 – Comparison of LX100 and MAGPIX Calculated Biomarker Concentrations. Multiplex reactions of HepG2 cell supernatant were assayed for the indicated cancer biomarkers along with standard curves in parallel using LX100 and MAGPIX readers. Determined concentrations from both readers are compared.

Inter-Assay Repeatability

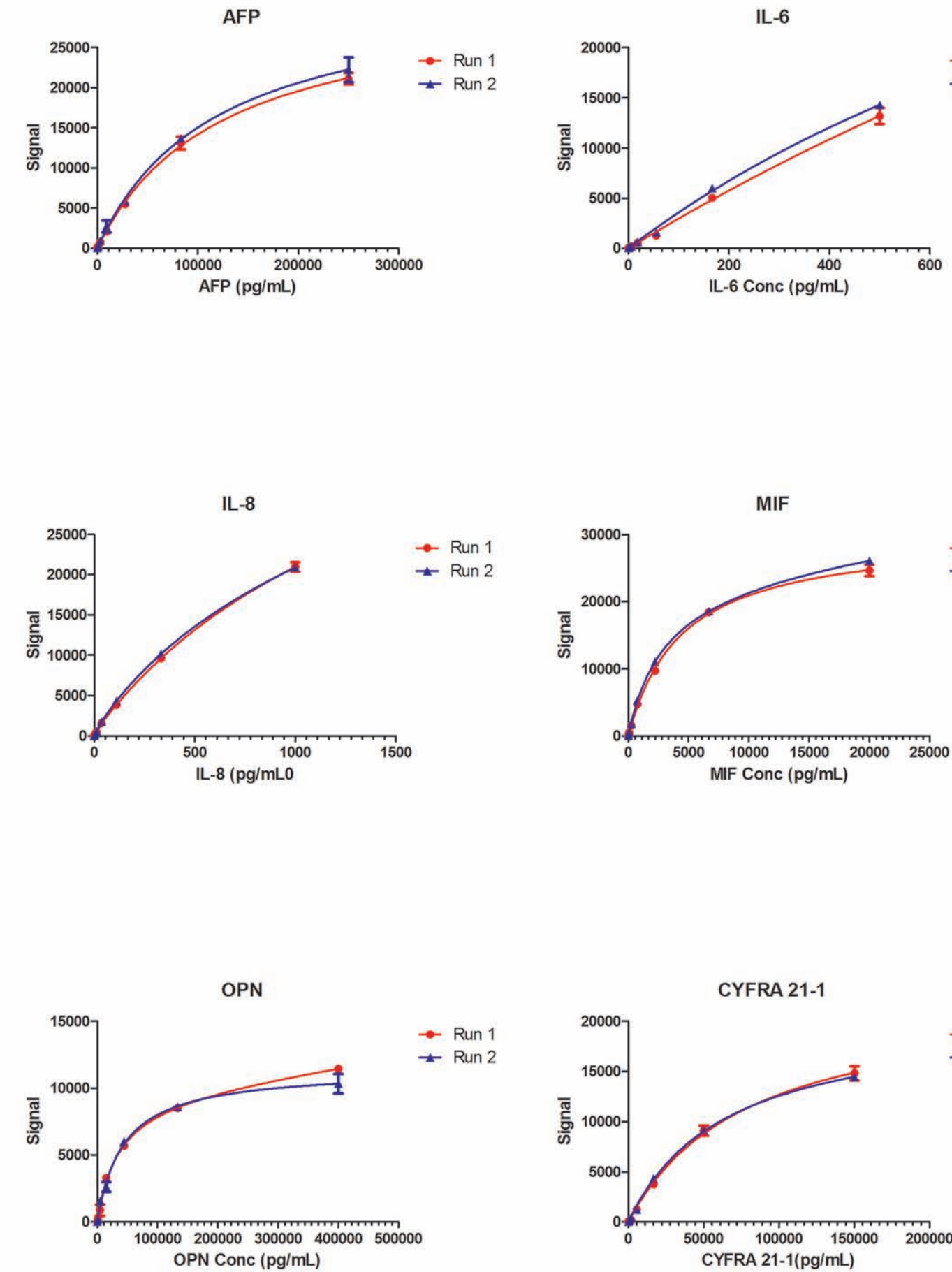


Figure 7 – Comparison of LX100 and MAGPIX Calculated Biomarker Concentrations. Multiplex reactions of HepG2 cell supernatant were assayed for the indicated cancer biomarkers along with standard curves in parallel using LX100 and MAGPIX readers. Determined concentrations from both readers are compared.

Conclusions

- ELx50 Bead Washer is Capable of Automating Assay Processing steps for both LX100 and MAGPIX Readers
- The EMD Millipore Multiplex Cancer Biomarker Assay kit is Capable of Measuring at least 22 Different Biomarkers Simultaneously
- MAGPIX and LX100 Readers Provide Equivalent Results at Low and High Signal Values
- MAGPIX Reader Demonstrates Very Good Inter-Assay Repeatability
- MAGPIX Reader is Capable of Providing Quantitative Results