Equivalent Analytical Performance Between the New MAGPIX® System and the Luminex® 100/200™ System

Introduction

Luminex launched its first xMAP Technology based instrument, the Luminex® 100™, in 1999. This system combined flow cytometry with differentially dyed microspheres to simultaneously analyze up to 100 different targets in a single sample. In 2006 Luminex updated the original instrument design and launched the new system as the Luminex 200™. There are currently more than 8,000 Luminex 100/200 instruments in life science research and clinical diagnostic laboratories worldwide. The FLEXMAP 3D® instrument, a system that enables higher throughput and can analyze up to 500 targets per sample, was launched in 2009. To cater to academic and smaller labs concerned about complexity and cost of these high-throughput instruments, Luminex developed the MAGPIX instrument, which was launched in July 2010.

The MAGPIX instrument is a compact fluorescent-based detection system capable of simultaneously measuring up to 50 analytes in a single well of a microtiter plate. Unlike the Luminex 100/200 and FLEXMAP 3D systems, the MAGPIX system is not based on flow cytometry, but instead uses light-emitting diodes (LEDs) for excitation and a CCD camera for detection. This easy-to-use instrument comprises a fluidics system, consisting of a syringe pump, a sample probe, a sample valve; a sample tray that is compatible with most 96-well plate formats; on-board Drive Fluid and waste containers that include liquid level sensors to alert users when the system is running dry or risks overflowing; and the optics module, which includes the sample chamber, magnet, LEDs, and CCD camera.

The New MAGPIX System —Cost-Effective xMAP Technology for Smaller Labs

Like the Luminex 100/200 system, the MAGPIX system uses color-coded magnetic microspheres as the substrate on which assays are performed and offers the option to multiplex, enabling researchers to investigate a large number of analytes using a minimal amount of sample. The MAGPIX is an out-of-the-box, user-installed system that includes the MAGPIX instrument, a computer, and software. The MAGPIX instrument is approximately the size of a desktop PC, and therefore, occupies minimal bench space.

A previous study performed by the Thomas Joos Laboratory of the Natural and Medical Sciences Institute (NMI) at the University of Tubingen used parallel assays to compare a sandwich immunoassay on ELISA to the MAGPIX system (xMAP Technology Technical Note: Overcoming the Cost and Performance Limitation of ELISA with xMAP Technology). That study showed that the MAGPIX instrument offers all the benefits of ELISA in addition to higher throughput, increased flexibility, reduced sample usage, and lower costs.

One of the most widely used applications of xMAP Technology is multiplexed immunoassays. In the current study, Radix BioSolutions (Georgetown, TX) performed a comparison of the Luminex 100/200 and MAGPIX systems. Standard curves and normal human serum samples were analyzed using the Bio-Plex Pro™ Assay (Bio-Rad) and the MILLIPLEX® MAG Kit Human Cytokine/Chemokine Panel (Millipore). Parallel assays were performed using the same antibodies and recombinant standard proteins for both instruments. For ease of comparison, identical material preparations were used. In order to compare the performance of the two instruments when measuring real experimental samples, unknown protein and nucleic acid samples were also analyzed. The intra-run and inter-run reproducibility, and dynamic range, and accuracy of the systems were compared.

Reproducibility of the MAGPIX Instrument is Comparable to the Luminex 100/200 Instrument

The Bio-Plex Pro™ Assay and the MILLIPLEX® MAG Kit Human Cytokine/Chemokine Mag Bead Panel were used for the comparison of protein analysis capabilities of the MAGPIX and Luminex 100/200 Instruments. These are two widely used research immunoassays built on xMAP Technology. For the Bio-Plex Pro™ Assay, seven standard concentrations of a four-fold serial dilution of reconstituted human cytokine standard were analyzed. Milliplex® MAP Panel analysis was

performed using the same cytokines; however, six standard concentrations of a five-fold serial dilution were used. Both assays were performed according to the manufacturer's instructions. Human serum samples from normal subjects, obtained from Bioreclamation, were also analyzed to compare results from actual samples using the two instruments. All analyses were performed in triplicate.

The intra-run reproducibility for each system was calculated as the coefficient of variation (CV; expressed as a percentage) of replicates. The range of analyte concentrations measured was 0.02–7775.50 pg/mL for the Bio-Plex Pro™ assay and 3.2–10,000 pg/mL for the MILLIPLEX® assay. The MAGPIX system demonstrated comparable reproducibility to the Luminex 100/200 system with typical CV values of 2.94–18.17% (Bio-Plex Pro™) and 1.15–20.92% (MILLIPLEX®) over the concentration range analyzed. Corresponding CV values for the Luminex 100/200 instrument were typically 0.88–20.95% (Bio-Plex Pro™) and 0–21.29% (MILLIPLEX®).

The inter-run reproducibility for each instrument was determined as the CV for three separate runs. There was high concordance between runs for both instruments with typical CV values of 0.61–14.29% (BioPlex Pro™) and 0.44–25.45% (MILLIPLEX®) for the MAGPIX system; typical CV values for the Luminex 100/200 system were 0.12–25.39 (BioPlex Pro™) and 0.22–20.64 (MILLIPLEX®).

To compare nucleic acid analysis data obtained from the two instruments, a Radix custom-designed assay was used. Briefly, a standard curve for four complimentary oligos was created, using a starting concentration of 20 ηM of each oligo to prepare a half log dilution series (100 µL + 216 µL Tris-NaCl) of seven standards that were hybridized to magnetic beads and allocated unique identifiers, 12, 15, 57, and 72. Streptavidin, R-phycoerythrin conjugate (SAPE; Invitrogen) was diluted with Tris-NaCl to a final concentration of 20 µg/mL. Magnetic beads were diluted in Tris-NaCl to a final concentration of 1.25 x 105 mL/set. Five microliters of each standard was combined with 25 µL of bead mix in the wells of a solid support microtiter plate and sealed. The DNA was bound to the beads according to the manufacturer's instructions, and then analyzed in the appropriate reader. Both instruments showed consistently low intra-run CV values of 1–15% (MAGPIX) and 1–13% (Luminex 100/200).

Inter-run CV values were 1–24% for the MAGPIX instrument and 5–21% for the Luminex 100/200 instrument (data not shown).

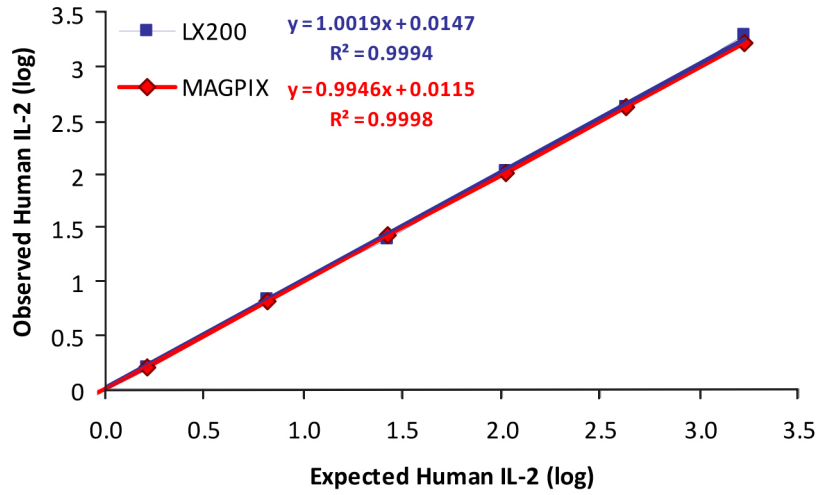
Performance of the MAGPIX Instrument is Comparable to the Luminex 100/200 Instrument

The data obtained from the standard concentrations of reconstituted cytokines were also analyzed to compare the accuracy, sensitivity, and dynamic range of both instrument systems for analyzing proteins using the Bio-Plex Pro™ Assay and the MILLIPLEX® MAG Kit Panel.

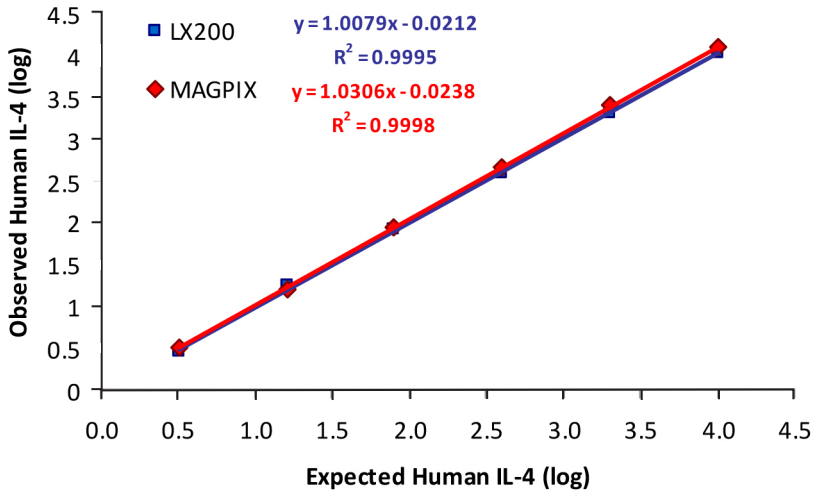
Both assays performed consistently over 5 standard concentrations (Figure 1), giving measurements close to the expected values (Figure 1). Actual percentage recovery of the standards was 90–116% for the BioPlex Pro™ and MILLIPLEX® MAG assays, well within the typical range of 80–120%, demonstrating the high accuracy of the instruments (data not shown).

Figure 1: Observed vs. Expected Concentrations of Bio-Plex Pro™ and Milliplex® Assays Performed on MAGPIX and Luminex 200 Instruments

A: BioPlex Pro™ Assay

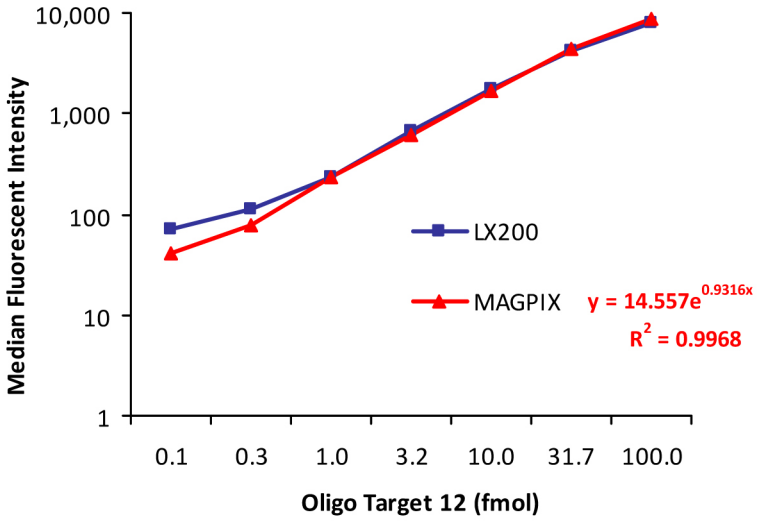


B: Milliplex® Assay



For nucleic acid analysis, measurements obtained using the MAGPIX instrument showed excellent concordance with those obtained with the Luminex 200 instrument (Figure 2). Comparing the sensitivity of the two instruments for nucleic acid analysis demonstrated that both instruments routinely detected as little as 0.1 fmol DNA.

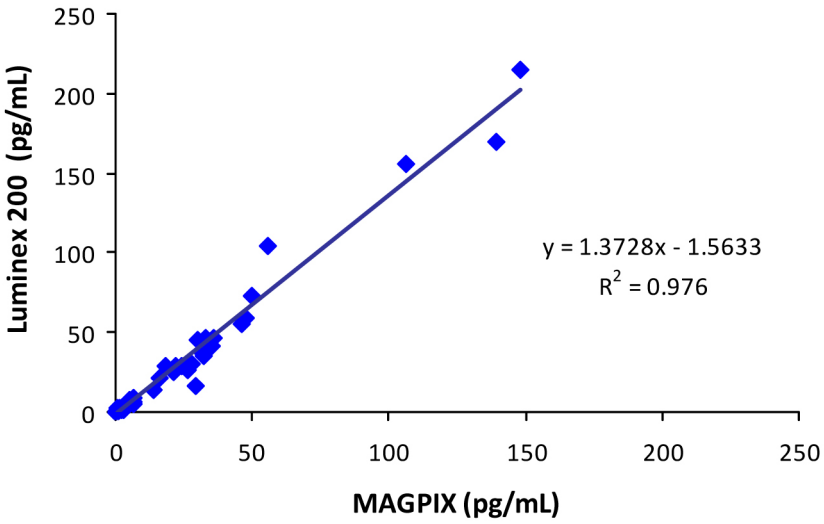
Figure 2: Observed Correlation Between MAGPIX and Luminex 200 Instruments for Nucleic Acid Analysis



Measurement of Unknown Samples

To compare the identification of analytes in unknown samples by the two instruments, 16 normal human serum samples were analyzed using the BioPlex Pro™ and MILLIPLEX® assays. Both instruments detected equivalent amounts of the various cytokines in each sample, demonstrating excellent concordance between the two instruments. Data obtained from the MILLIPLEX® assay are shown (Figure 3).

Figure 3: Observed Concordance Between MAGPIX and Luminex 200 Instruments When Measuring Unknown Samples.



This study demonstrates that the MAGPIX system delivers comparable results to the Luminex 200 system. The lower complexity and reduced costs involved in running the MAGPIX system make it especially suitable for bridging the gap between singleplex and multiplex analysis of proteins and nucleic acids.

The MAGPIX system is versatile, delivering analytical performance equal to or better than standard ELISA methods and enabling analysis of both proteins and nucleic acids. The system can be used with pre-optimized test kits or laboratory-developed assays. Protocols run on the MAGPIX system are comparable to ELISA and much simpler than western blot; additionally, they are more cost-effective than the ELISA, making them suitable for medium-throughput use.

Conclusion

The MAGPIX system offers all the benefits of the ELISA with the added value of automation, increased throughput and flexibility, reduced sample usage, and lower costs. This study shows that the new MAGPIX system performs as well as the well-established Luminex 100/200 system, demonstrating similar reproducibility and sensitivity to the Luminex 100/200 system for the analysis of both pre-optimized and laboratory-developed tests. The use of a CCD camera in the MAGPIX, rather than flow cytometry, reduces the complexity of the instruments and enables easy adoption of the technology by even the smallest research labs. All of the benefits of using xMAP Technology and magnetic beads are retained—reduced costs and labor by easy multiplexing, smaller sample requirements and faster time-to-results enabled by the favorable reaction kinetics of the liquid bead array approach, and focused, flexible multiplexing capabilities. Similar to the Luminex 100/200 system, the MAGPIX system can be used for a wide variety of applications, including protein expression profiling, focused gene expression profiling, and disease testing. The data obtained from the Bio-Rad and Millipore kits also demonstrate that the MAGPIX system is compatible with existing kits available for the Luminex 100/200 system and delivers comparable results.

References

Carson, R. T. and Vignali, A. A. (1999). Simultaneous quantitation of 15 cytokines using a multiplexed flow cytometric assay. J. Immunol. Methods 227: 41–52.

Luminex Corporation. (2010). Overcoming the Cost and Performance Limitations of ELISA with xMAP® Technology. [White Paper]

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