Luminex®

MAGPIX® Provides Equivalent Performance to the Luminex® 100/200™ in an HPV Vaccination Trial

Introduction

The MAGPIX instrument is a compact fluorescence-based detection system capable of simultaneously measuring up to 50 analytes in a single well of a microtiter plate. Like the Luminex 100/200* systems, the MAGPIX system uses xMAP® technology. This technology is based on the use of color-coded magnetic microspheres as the solid support 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 also similar to previous Luminex instruments in that it is an open system that allows researchers to build custom multiplex assays which transfer easily between all of the Luminex systems.

Unlike the Luminex 100/200, the MAGPIX system is not based on flow cytometry and laser-induced fluorescence, but instead uses light-emitting diodes (LEDs) for excitation and a CCD camera for detection. This new design reduces the complexity of the instrument and enables easy adoption of xMAP technology by even the smallest laboratories. This Technical Note demonstrates equivalent performance to the Luminex 100/200 for the MAGPIX with a previously designed custom HPV serology assay.

xMAP Technology has Played a Key Role in HPV Serology Research

Genital infection with human papillomavirus (HPV) is one of the most common sexually transmitted diseases, and persistent infection can lead to cervical cancer in women. HPV serology is complex, because there are over 100 known papillomavirus types, and infection and disease lead to distinct type-specific antibody responses. Luminex xMAP technology has played a critical role in developing an understanding of HPV serology that eventually led to the development of an effective vaccine (Gardasil®, Merck and Co., Inc.), with numerous papers on HPV serology having referenced xMAP technology. For example, several evaluations of the HPV virus-like particle (VLP) vaccine have been conducted using an xMAP serology assay to determine its potency (Emeny et al. 2002) and assess its safety and immunogenicity in young women (Villa et al. 2006) as well as in HIV-infected children (Levin et al. 2010).

An xMAP assay has also been developed by Waterboer et al. (2005) to enable the simultaneous detection of antibodies against up to 100 *in situ* affinity–purified recombinant HPV proteins. Spectrally distinct bead sets, each carrying one particular HPV antigen fused to glutathione S-transferase (GST), were mixed, incubated with serum, and analyzed for the presence of antibodies on the Luminex 100 system. The dynamic range of the assay covered 1.5 orders of magnitude, precision was excellent (Coefficient of Variation ≤5.4%), and results on clinical samples showed high concordance with ELISA (κ=0.846) and increased detection of weak antibody responses.

This xMAP assay for HPV antibodies was subsequently used by Kaufmann et al. (2007) to measure response to a novel vaccination approach utilizing chimeric virus-like particles (CVLPs) that are able to induce L1- and E7-specific cytotoxic T lymphocytes. A randomized, double blind, placebo-controlled clinical trial was conducted in 36 HPV type 16 (HPV16) mono-infected high grade cervical intraepithelial neoplasia (CIN 2/3) subjects. The results using the Luminex 100 platform demonstrated that antibodies against HPV L1 and E7 antigens, as well as cellular immune responses against both proteins, were induced.

MAGPIX Provides Equivalent Results to Luminex 100 in the HPV Vaccination Trial

A recent study performed by the Waterboer research group at the German Cancer Research Center (Deutsches Krebsforschungszentrum, DKFZ) in Heidelberg repeated the analysis of the serum samples taken in the HPV vaccination trial, using the MAGPIX

**The Luminex 200 has replaced the Luminex 100 and the two instruments can be considered to be equivalent in performance.*

system. This data was then compared to the data generated in the original HPV vaccination trial, completed almost six years earlier. In the original vaccination trial, 2 different groups received 75 µg or 250 µg of CVLP vaccine per vaccination at four time points (0, 2, 6 and 12 weeks), and these were compared to placebo. This study was designed to give a first impression of the safety profile of a CVLP vaccination. The duration of the study was 24 weeks, and serum samples were taken at 0, 2, 4, 8, 14 and 24 weeks for analysis. Antibodies with high titers against HPV-16 L1 and low titers against HPV-16 E7 were induced.

The testing performed in the MAGPIX equivalence study used recombinant GST fusion proteins of the HPV-16 L1 antigen, HPV-16 E7 antigen and GST alone as a control, as did the Luminex 100 study. Sera were tested at 1:100 final dilution. Placebo follow-up sera (12 subjects in the placebo group x 5 follow-up visits per subject = 60 total sera) served as negative controls, and they were used to determine cut-offs, which were calculated as the mean plus 3 standard deviations. Repeated measurements of different GST fusion protein-loaded bead sets yielded excellent comparability, with a median coefficient of variation (CV) of 1.5%, and a range of 1.1-1.9%. Reproducibility of triplicate samples was also excellent, with a median CV of 2.1% and a range of 2.0-2.5%.

Initial comparison of the MAGPIX data with the original Luminex 100 data showed substantial, but insufficient correlation (Figure 1). However, the MAGPIX system may have a wider dynamic range, resulting in the "bending" of the correlation curve at the lowest and highest antibody concentrations. This effect may also be due to the fact that the assay has been improved over the intervening six years, and some components of the assay have simply changed, due to changes in vendors.

In spite of these changes, stratification of the data by study group demonstrates that the L1 antibody patterns for the Luminex 100 and the MAGPIX data were almost identical for all three groups (Figure 2). Only one of the 12 placebo subjects showed any significant levels of the antibody, and these were present from the first visit, due to the pre-existing HPV16-induced CIN2/3 lesions. Both MAGPIX and Luminex 100 data showed a steady increase with time in antibody levels for all subjects in the low-dose group, and a more rapid increase for most subjects in the high-dose group. Most of the fluorescence readings for the low- and high-dose groups are significantly higher than those for any of the subjects in the placebo group. A striking difference across the two data sets is the fact that a much higher signal is often obtained on the MAGPIX platform versus the Luminex 100, particularly for the high-dose vaccine group. This may be due to a wider dynamic range, as suggested by Figure 1, or again it may be due to improvements to the assay within the past six years.

Figure 1. Correlation of Luminex 100 data versus MAGPIX data (HPV-16 L1 antibody). The Pearson correlation coefficient (R^2) for linear regression was 0.84, and the slope of the trend line was 1.28.

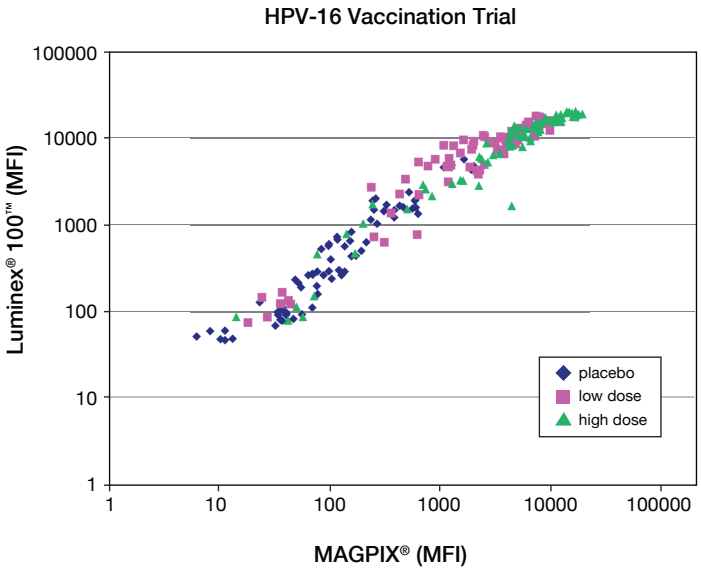
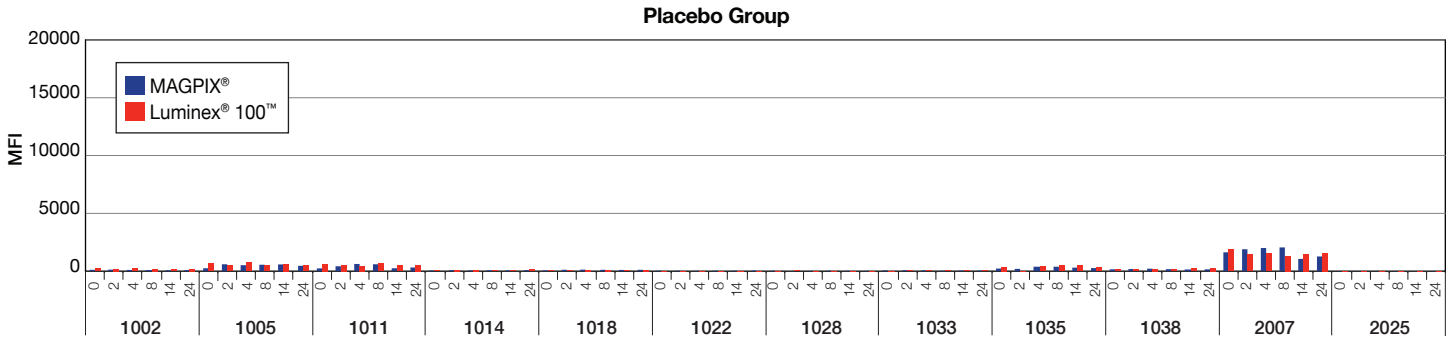
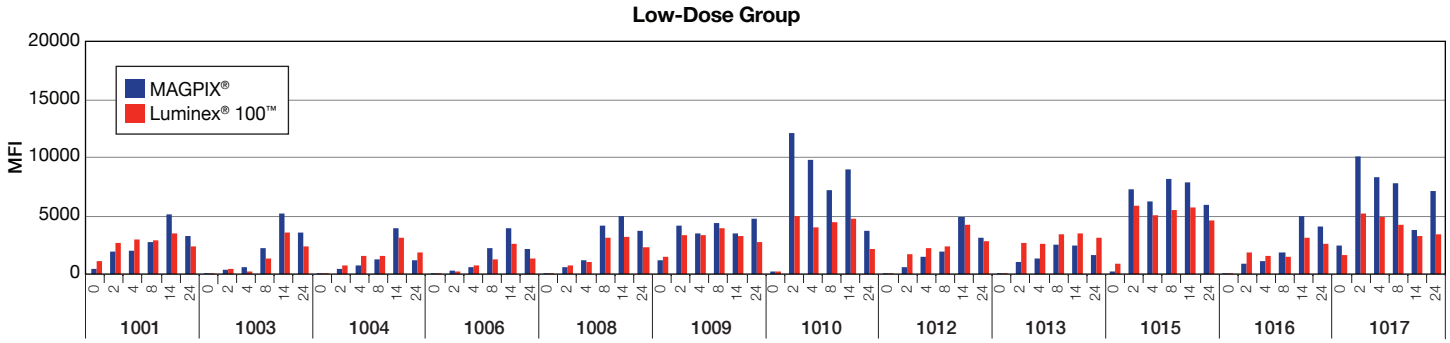
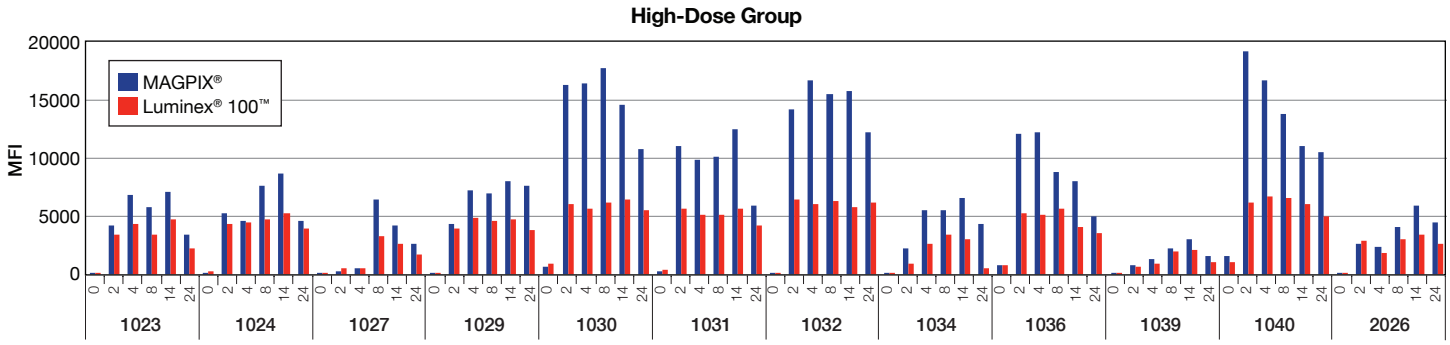


Figure 2. Comparison of Luminex 100 and MAGPIX data after stratification by study group (HPV-16L1). The 12 subjects in each group are identified by code numbers, and each of the six visits for each subject is displayed on the X-axis. The antibody response at each visit, for each patient, is displayed on the Y-axis and is represented by MFI (Median Fluorescence Intensity).







Conclusion

The Waterboer research group found the MAGPIX system to be a very useful tool for this study that performed as well as the Luminex 100 system for this application. They found that the MAGPIX could be put into operation without a service technician, and analysis time per well was identical to the Luminex 100 readings. Furthermore, the system performed very reliably in their hands, without a single measurement malfunction observed. Most importantly, the fact that the MAGPIX assay generated almost identical antibody response patterns to those seen in a study done six years earlier, and would have led the researchers to the same conclusions for the vaccination trial, is a dramatic demonstration of the equivalent performance of MAGPIX and Luminex 100 for this application.

The MAGPIX system offers all the benefits of the ELISA and other immunoassay formats with the added value of automation, increased throughput and flexibility, reduced sample usage, and lower costs. The use of LEDs and a CCD camera in the MAGPIX, rather than a laser and 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, including focused and flexible multiplexing capabilities. Reagent and labor costs are reduced by easy multiplexing and smaller sample requirements. Faster time-to-results is enabled by the favorable reaction kinetics of the liquid bead array approach. As is the case for the Luminex 100/200 system, the MAGPIX system can be used for a wide variety of applications, including protein expression profiling (VanDerMeid et al. 2011), focused gene expression profiling (Peck et al. 2006), and disease testing (Liu et al. 2011).

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