Comparison of Luminex® 200™ to MAGPIX® using the Poultry Serology Assay
Technical Notes
Background and Rationale:
Luminex® xMAP® Technology affords many advantages over traditional assay formats, namely the ability to test for multiple analytes simultaneously from the same sample, commonly referred to as “multiplexing”. At the heart of the xMAP Technology are the microspheres and the proprietary dyeing process that assigns each microsphere region a unique and specific spectral address, thus making multiplexing possible. Until recently, analysis of these microspheres required the sophisticated fluidics and optics of the Luminex 200® or FLEXMAP 3D® analyzers. With the launch of the MAGPIX® system, xMAP assays utilizing magnetic microspheres can be analyzed without the use of lasers or hydrodynamic focusing. The MAGPIX is a compact, robust, cost effective multiplexing system based on CCD imaging technology. Furthermore, the MAGPIX instrument was designed to yield equivalent results to the Luminex 200 instrument. Users can expect to see similar median fluorescence intensity (MFI) data from their assays, whether run on the MAGPIX or Luminex 200 system. To demonstrate equivalency between the systems, we conducted several experiments using a model quantitative serology assay based on the Poultry Serology Reagents.

In July 2010 Luminex completed development of set of multiplex Poultry Serology Reagents to aid in the measurement of antibody titers in production chickens. Both responsible animal welfare and profitable live production groups rely on using veterinary diagnostic kits to monitor immune response in vaccinated production flocks over their lifetimes. Flocks that over time demonstrate decreasing antibody presence (referred to as “titer”), can be boosted with another round of vaccination. The Luminex Poultry Serology Reagents can be used by customers to develop magnetic bead-based immunoassays designed to quantitated antibodies in chicken serum for Infectious Bursal Disease Virus (IBDV), Newcastle Disease Virus (NDV), Infectious Bronchitis Virus (IBV), and Reovirus (REO). The Poultry Serology Reagent Microsphere Mixture also contains two internal control microspheres that allow operators to verify critical reagent additions.

Veterinary diagnostic labs are susceptible to variable environmental conditions and historically have been funded at minimal levels. Therefore, providing reagent materials that can be run on a robust detection platform with low cost of ownership are critical factors for commercial success. The Poultry Serology Reagents are an excellent choice for use in this instrument comparison study.

Materials and Methods:
Materials used during this study included the Poultry Serology Reagent Microsphere Mixture, Sample Diluent, Wash Buffer, Detection Antibody, Streptavidin-Phycoerythrin (SA-PE) Conjugate, five Standards, Negative Control, Positive Control, and High Positive Control.

The Poultry Serology Microsphere Mixture is a suspension containing four viral antigen coupled microspheres and two antibody coupled microspheres. The antigens coupled to the microspheres are partially purified, chemically inactivated viral particles of IBDV, NDV, REO, and IBV and the antibodies are chicken IgY (IgY) and a rabbit anti-chicken (Rb Ab) antibody. The antibody coupled microspheres are intended to be used as internal assay controls and will confirm the addition of critical reagents. The rabbit anti-chicken coupled microsphere, this reagent ensures that the user has added sample. In the case of the chicken IgY coupled microsphere, ensures that the user has added detection antibody. These internal controls help elucidate false negatives caused by user error. MFI values greater than 7500 MFI for each internal control microsphere indicate successful reagent addition.
Twenty chicken serum samples were selected for testing. These samples include negative, low positive and high positive samples and originate from field exposure, vaccination, and experimental infection. Some samples are negative for the viral disease antibodies in this test but shown to be positive for antibodies to other common poultry diseases. All samples were previously characterized via a USDA recognized gold standard method and are described in Table 1.

Table 1: Expected Results for Chicken Serum Samples

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The Poultry Serology Reagent standards were created from a mixture of four monovalent antisera produced by experimental infection. The antisera were combined in a specific ratio and diluted to create five standards, which are supplied to the user at working concentration and do not need to be diluted further. Expected values for the standards were determined from gold standard titer values provided by the antisera supplier and are reported as “titer” units. These five reference concentrations cover the entire dynamic range of the Luminex 200 instrument and control for environmental and operator influenced assay variance, thus enhancing precision. The reference concentrations create a five point standard curve based on logistic 4-parameter regression. Unknowns can be fit to the standard curve by the xPONENT® software allowing relative quantitation. The 4-parameter logistic regression algorithm provides an accurate model of immunoassay binding kinetics, thereby giving the user confidence in the integrity of the interpolated data produced from the curve (See Luminex Technical Note: xPONENT® Logistic Curve Fitting).

Procedure:
Two identical assay plates using the same diluted samples were prepared with the Poultry Serology Reagents under the same conditions. One plate was analyzed on the Luminex 200 and the other was analyzed on the MAGPIX instrument.

The Negative Control, Positive Control, High Positive Control and twenty samples were diluted 1:500 and run in duplicate on each plate. The Standards are provided ready to use and did not require further dilution. Standards were also run in duplicate on each plate.
Figure 1: Plate Layout:

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The Poultry Serology Reagents were used with the Luminex “SAMPLE PROTOCOL FOR WASHED SEROLOGICAL ASSAY USING MAGNETIC MICROSPHERES” found on the FAQ section of the Luminex website (http://www.luminexcorp.com/support/Magnetic%20Microspheres/index.html).

- 50ul of Microsphere Mix was added to each test well
- 50 µl of diluted sample/ diluted Control/Standard was added to the appropriate test well
- The plate was incubated at room temperature for 1hr on the plate shaker at 800rpm
- The plate was then washed with 100ul of wash buffer two times in a plate washer
- 100ul of detection antibody was added to each test well
- The plate was incubated at room temperature for 30min on a plate shaker at 800rpm
- The plate was then washed with 100ul of wash buffer two times in a plate washer
- 100ul of SA-PE Conjugate was added to each test well
- The plate was incubated at room temperature for 30min on a plate shaker at 800rpm
- The plate was then washed with 100ul of wash buffer two times in a plate washer
- 100ul of wash buffer was added to resuspend the beads and the plate was placed on a plate shaker at 800rpm for 10 seconds.
- The plate was placed in the instrument and read.
Results:
Figures 2 and 3 show the standard curves for each analyte from the MAGPIX and Luminex 200 analysis respectively. The xPONENT 3.1 software on the Luminex 200 instrument and the xPONENT 4.1 software on the MAGPIX instruments fit the standards by the same logistic 4-parameter algorithm. The curve shapes are very similar between the two systems and the coefficient of correlation for each curve is near 1.0.

Figure 2: MAGPIX Standard Curves for the Poultry Serology Reagent Standards

Figure 3: Luminex200 Standard Curves for the Poultry Serology Reagent Standards
The S/P Ratio, or Sample to Positive Ratio, is the poultry industry metric for determining antibody presence in flocks. This ratio is used to determine whether a sample tests as positive or negative by comparing it to a cut-off number. This cut-off value can be determined by the customer or by the assay manufacturer with regulatory approval. The S/P Ratio is defined mathematically as the \( \frac{\text{Sample MFI} - \text{Average Negative Control MFI}}{\text{Average Positive Control MFI} - \text{Average Negative Control MFI}} \). When analyzed by comparison scatter plot, the coefficient of correlation between the S/P Ratios produced by the Luminex 200 and the MAGPIX instruments for the same samples is greater than 0.99, indicating strong correlation. Though no S/P Ratio cut-off values are supplied with the Poultry Serology Reagents, if the data is scored using the cut-off values from commercially available ELISA kits (greater than 0.2 is positive) as shown in Table 2, the sample results match the expected results shown in Table 1. Furthermore, the results are scored correctly regardless of which instrument platform was used for the analysis.

Scatter plots with linear regression were performed on the Standards titer, median MFI and the S/P Ratio data as a means to graphically demonstrate the similarity or dissimilarity between the LX200 and MAGPIX instruments. The R is presented as a measure of the closeness of the linear relationship between the two parameters plotted. It is generally accepted that the closer the correlation constant is to 1, the closer to a linear fit between the two data sets, meaning the more similar the data sets are to each other. The R values for the titer of the standards, median MFI and S/P Ratio are all over .99. (Fig. 4, 5, and 6)

Figure 4: S/P Ratio Correlation of Samples Analyzed by the Luminex 200 and MAGPIX Systems
Table 2: S/P Ratio for Samples Analyzed Using MAGPIX or Luminex 200 Instruments.

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Comparison scatter plots with best fit lines were generated using the actual, calculated titer of the Standards and median MFI of the samples, as shown in Figures 4 and 5 respectively. The relationship in titer calculated from the Luminex 200 run and the MAGPIX run was very similar, resulting in a linear best fit with a correlation coefficient and slope near or equal to 1.0 (Figure 4). The R values for the titer of the standards, median MFI and S/P Ratio are all over .99. (Figures 4, 5, and 6)
Figure 5: Correlation of Poultry Serology Reagent Standards’ Titer between MAGPIX and Luminex 200 Analysis

Figure 6: Correlation of Chicken Serum Sample MFI between MAGPIX and Luminex 200
Summary:
The data from this study show the Luminex 200 and MAGPIX correlate well with each other and produce accurate data when compared to gold standard methods. MFI and S/P Ratio data show that the MAGPIX reads the Poultry Serology Reagents nearly identically to the Luminex 200.