Luminex Technology- Innovative Flow-and-Bead Based Technology Measuring Multiple Analytes Simultaneously in a Single Reaction Well

In simple terms, Luminex is a fluorescence covalent microbead immunosorbent assay. Up to 500 analytes can be multiplexed in a single well. Luminex x-MAP technology, the one most commonly used, is a flexible, open architecture design that can be configured to perform a wide variety of bio-assays.

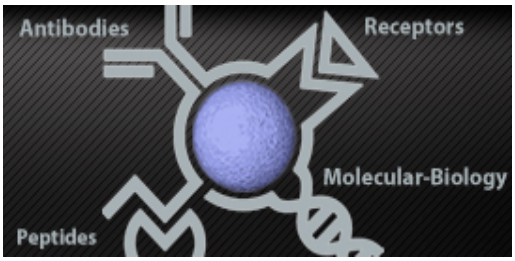

Figure 1.0

Luminex incorporates three types of assay platforms currently[i]:

1) **Multiplexed Bioassay capabilities: This is through the platform of microsphere-based open architecture-driven** xMAP Technology. Up to 500 analytes can be analyzed per microwell.
2) **Clinical nucleic acid assays: This is powered by** Luminex's patented universal tag system based-**xTAG** Technology**.** Up to 100 DNA tests can be multiplexed in one microwell.
3) Real-time and multiplex **PCR-based assays: This is based on** Luminex's patented **Multicode technology.** The unique features of this technology are: flexibility, probe-free, single tube multiplex real-time PCRs.

xMAP technology[ii]

The concept of Luminex's multiplex bead-based immunoassays, such as the commonly used Luminex xMAP technology, is based on sandwich bioassays. The difference being that, instead of a solid phase binding of capture antibodies, and then subsequently analytes, as in traditional ELISAs, simultaneous measurement of multiple analytes using a catalog of antibodies bound to differentially color-coded beads is utilized. Each bead is identified by the unique wavelength it emits when excited by the first laser. Quantitation is accomplished by a sandwich assay using a fluorescently labeled detection antibody (versus HRP-coupled detection antibody in most ELISAs) with affinity to the specific analyte captured by the bead-coupled antibody beads. Excitation by a second laser quantitates the bound detection antibody. Multiple readings on each bead set further validate the results. Using this process, xMAP Technology allows multiplexing of up to 500 unique bioassays within a single sample, both rapidly and precisely.

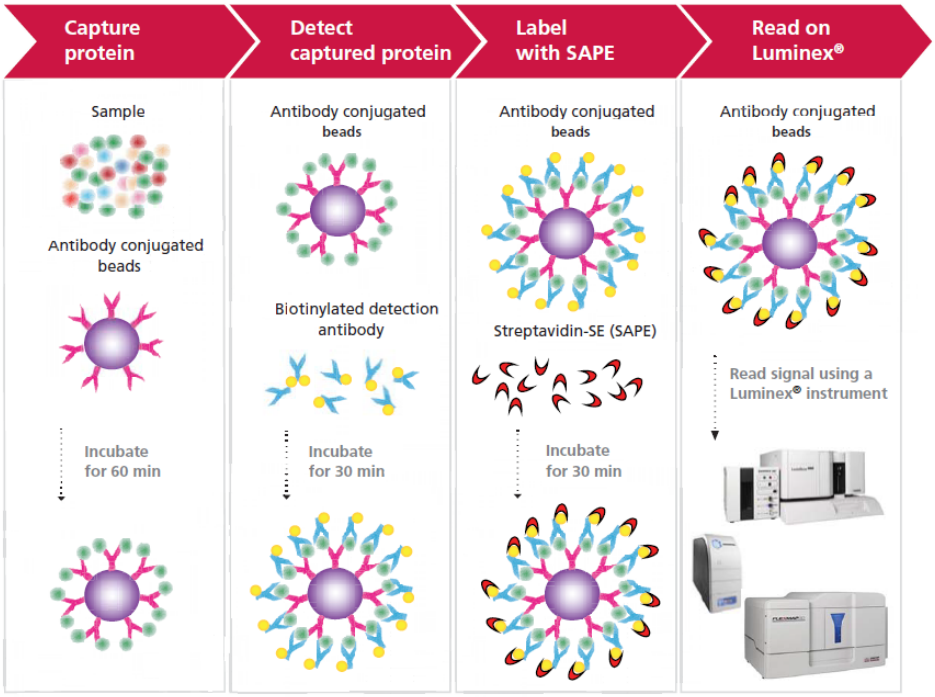

Figure 2.0

[iii] **From the** Procarta® Immunossay User Manual

The advantages of Multiplexed bead-based immunoassays, which make it an attractive option to other bioassays are speed and high-throughput, sample size, accuracy and reproducibility, versatility, flexibility, and powerful software support via the xPONENT® software.

For GLP quality level, Luminex software is linked to the 21CFR Part 11 module, and is used for GLP validation

Figure 3.0

Flexmap 3D Luminex instrument, with xPONENT software

Can multiplex up to 500 analytes

Luminex versus ELISA

Luminex technology has emerged in the past 15 years that offers the benefits of the ELISA, but also enables the added value of higher throughput, increased flexibility, reduced sample volume, and low cost with the same workflow as ELISA ,and can be applied to both protein and nucleic acid applications.

While widely utilized, ELISA can have limitations, which Luminex can overcome.

1) Sample volume: A solid-phase ELISA typically performed in a 96-well microplate, requires a relatively large amount of sample. Since the Luminex microbeads have the capture antibody immobilized on their much smaller surface area compared to a microplate well, smaller sample volumes are required. This is especially important when working with limited sample types such as cerebrospinal fluid, synovial fluid, tears, mouse serum, etc .

2) Non-specific binding: ELISAs can be limited by non-specific binding and increased background due to large surface area of the individual ELISA microplate wells and the hydrophobic binding of capture antibody. Since the Luminex microbeads have the capture antibody immobilized on their much smaller surface area compared to a microplate well, non-specific binding is reduced. Thus, Luminex can be an alternative for ELISAs which are limited from non-specific binding and high background.

3) Flexibility: The suspended Luminex microbeads allow for assay flexibility in a singleplex or multiplex format.

4) Cost: Cost comparisons of sandwich ELISA versus Luminex, performed at The Thomas Joos Laboratory at Natural and Medical Sciences Institute (NMI) at the University of Tuebingen (Tübingen, Germany), showed that costs of a Luminex MAGPIX assay were less than half the cost of the standard ELISA, utilizing the same pair of antibodies and the same recombinant standard. At the cost of a high performance ELISA plate reader, researchers can now obtain more information from less sample volume using a similar workflow to ELISA using the new MAGPIX system. Cost of bioassays is reduced when using Luminex based bioassays, because there is less capture antibody used due to the smaller surface area.

5) Time and labor costs: The total assay time is similar for both assays, while multiplexed Luminex assays have less decreased labor costs over a traditional singleplex ELISA.

6) Analytical performance: The Thomas Joos Laboratory at NMI carried out analytical comparisons between ELISA and Luminex. Starting at 10,000 pg/ml with a total of seven standard concentrations, both assays performed over the range of 14 to 10,000 pg/ml, with the MAGPIX Luminex assay having a more linear range than the ELISA. For this particular assay, The MAGPIX assay had a two-fold more

sensitive LOD and was five-fold more sensitive for LOQ, than the ELISA. The reason for the better performance of x-MAP based assays is that, firstly, the xMAP assays are based on direct fluorescence detection as opposed to colorimetric detection mediated by an enzyme, resulting in better sensitivity. Secondly, capture antibodies have higher avidity and lower background due to covalent coupling to microbeads as opposed to passive coating of the ELISA plates. This leads to a higher density of capture antibody per surface area and the capture antibodies will not wash off during the assay.

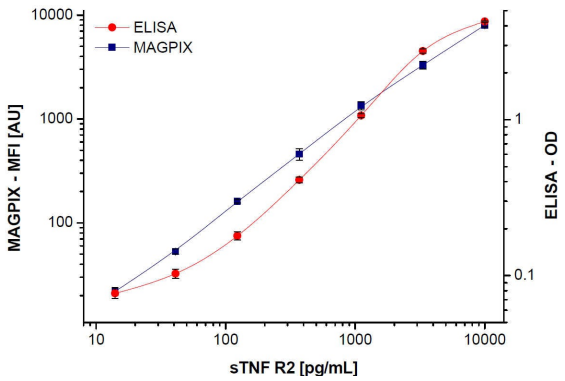

Figure 4.0

Applications of Luminex: A variety of flavors

We use Luminex-based assays to study oncological (cancer) biomarkers, multiplex cytokine and chemokine analysis of cell culture supernatants and tumor cell lysates, and Quantigene branched DNA analysis of tumor samples, among others. Quantigene analysis is a bead based quantitative gene expression analysis, which facilitates simultaneous and quantitative detection of 3 to 30 target-specific DNA or RNA molecules in different sample types. A major advantage of Quantigene is the ability to detect and quantify DNA and RNA copies in formalin fixed paraffimn embedded tissue (FFPE).

Luminex-based assays have been also been used for the following research areas, and should be considered as an option to other bioassays in these study areas, especially if high-throughput and multiplexing are required:

1) **Custom-designed assays:** To suit a variety of applications and research areas and needs
2) **Clinical Diagnostics:** Allergy Testing, Autoimmune Disease, Cystic Fibrosis, HLA Testing, Infectious Disease, Pharmacogenomics, Respiratory Viral Testing, Vaccine Testing
3) **Life Science Research:** Alzheimer's, Cancer Markers, Cardiac Markers, Cellular Signaling, Cytokines, Chemokines, & Growth Factors, Gene Expression Profiling, Genotyping, Endocrinology, Isotyping, Matrix Metalloproteinases, Metabolic Markers, Neurobiology, Transcription Factors/Nuclear Receptors
4) **Biosurveillance:** Biodefense, Food Safety and Animal Health

Comparative Technologies

In conclusion, xMAP Technology can offer all the benefits of the ELISA with the added value of higher throughput,

increased flexibility, reduced sample and lower costs with the same workflow as ELISA. Numerous studies have performed comparisons of the two technologies, as well as compared Luminex to other bioassays, like Mesoscale Discovery (MSD), and real-time PCRs (q-PCRs), and in many cases, have conclusively demonstrated the superiority and efficiency of Luminex-based assays over traditional ELISAs. An example of such a publication is: J Reprod Immunol. 2005 Aug;66(2):175-91.

Other studies have refuted the claim of Luminex that it is always superior to ELISA: Djoba Siawaya JF, Roberts T, Babb C, Black, Golakai HJ, et al. (2008) An Evaluation of Commercial Fluorescent Bead-Based Luminex Cytokine Assays. PLoS ONE 3(7): e2535.

Other papers recommend the use of multiple methods, not just Luminex, for confirming results, concluding that multiplex methods aren't as superior as singleplex ELISAs: Clin Vaccine Immunol. 2011 August; 18(8): 1229–1242. Multisite Comparison of High-Sensitivity Multiplex Cytokine Assays.

This multisite comparison suggests that current multiplex assays vary in their ability to measure serum and/or plasma concentrations of cytokines and may not be sufficiently reproducible for repeated determinations over a long-term study or in multiple laboratories but may be useful for longitudinal studies in which relative, rather than absolute, changes in cytokines are important

Additionally, studies have suggested that there will not necessarily be a good correlation between ELISAs and multiplexed Luminex bioassays for all cytokines tested[iv]. While with some cytokines, a good correlation is seen between the two methods, especially for the ones found at high concentrations in that particular matrix, depending on the analyte tested, the group performing the comparison, and also the matrix tested, a correlation can or cannot be seen. This will depend on multiple confounding factors, peculiar to that assay.

In conclusion, assay development utilizing this flexible, versatile, multiplexing technology is not unproblematic, or a complete replacement for ELISAs, as suggested by Luminex brochures, and other Luminex marketing resources. The applicability of this powerful technology will depend on multiple factors, including the cytokine to be quantified, the matrix to be tested, the limits of detection and quantitation, and the capture and detection antibody pairs. However, the advantages of a unique combination of high-throughput, multiplexing, and small sample size, makes this bioassay method a very attractive option to other, more traditional, bioassay methods.

Advantages of xMAP technology over other high density or high throughput technologies

The unique and two-pronged benefits of Luminex, unmatched by any other popularly used application, is the ability to provide high density and high throughput at the same time. Traditional ELISA, real-time PCR and other technologies that excel at high-throughput

applications (greater than 1000 samples per day) lack the ability to multiplex more than 5 tests at a time. On the flip side, microarray technology excels in high-density screening (greater than 250-plex tests) — but lacks the reproducibility needed for high-throughput applications. For applications requiring a throughput of up to 1000 samples per day, multiplexing from one to 500 tests per sample, xMAP Technology stands virtually alone.

[i] http://www.luminexcorp.com/TechnologiesScience/index.htm
[ii] http://www.luminexcorp.com/TechnologiesScience/index.htm
[iii] http://www.panomics.com/downloads/UM10482_Procarta_Poly_Manual_RevD_110406.pdf
[iv] http://iti.stanford.edu/research/documents/Luminex-performance-charWeb5-7-10.pdf

About the Author:

Dr. Sonal Gupta is a Senior Development Scientist in Research and Development, at Cambridge Biomedical. She is a cell biologist and immunologist with expertise in developing various assays based on techniques such as Luminex, flow cytometry (FACS), ELISA, Mesoscale Discovery (MSD), immunohistochemistry, and cell-based assay techniques such as ACEA, among others. She obtained her degree in Medicine from India, following which she pursued her PhD in cancer biology from the University of Bradford, in the United Kingdom. She has extensive and broad experience in oncology and inflammatory diseases, hematological cancers, skin disorders, cancer and melanoma research, and biologics and recombinant antibody technology, and has previously worked in these fields at the Massachusetts General Hospital, Abbott Bioresearch Center, and Shire Human Genetic Therapies.