Bio-Plex® multiplex system

tech note 6313

Overcoming the Cost and Performance Limitations of ELISA with Bio-Plex® Multiplex Readers

Introduction

For nearly half a century, immunoassays have been the primary source for detection of analytes of interest in biological samples for both life science research and clinical diagnostics. This began with the quest to measure insulin levels and culminated in the development of the radioimmunoassy (RIA) by Yalow and Berson (1960). The desire to use less hazardous detection methods than radioisotopes led to the development of the enzyme-linked immunosorbent assay (ELISA) by Engvall and Perlmann (1971). The advantages of ELISA, as evidenced by nearly 10,000 studies published per year utilizing the technique (Lequin 2005), are its ease of use, flexibility, low cost, and accessibility to any lab.

While widely utilized, ELISA has limitations. An ELISA is typically performed in a 96-well microplate and the wells are coated with capture antibody. In order to capture the antigen of interest from the sample, a relatively large amount of sample is required. The large surface area of the individual microplate wells and the hydrophobic binding of capture antibody can lead to nonspecific binding and increased background. Also, most ELISAs rely upon enzyme-mediated amplification of signal in order to achieve reasonable sensitivity.

Luminex xMAP Technology as an Alternative to ELISA

In the past 15 years, a new technology has emerged that offers the benefits of the ELISA, but also enables higher throughput, increased flexibility, reduced sample volume, and lower cost with the same workflow as ELISA. The Luminex xMAP technology (licensed to Bio-Rad by the Luminex Corporation) has been utilized in over 7,700 publications (www.luminexcorp.com/bibliography), and can be applied to both protein and nucleic acid applications. The suspended beads allow for assay flexibility in a singleplex or multiplex format. Since the beads have the capture antibody immobilized on their much smaller surface area, compared to a microplate well, smaller sample volumes are required and nonspecific binding is reduced (Carson and Vignali 2000). Smaller samples are important when working with limited sample types such as cerebrospinal fluid, synovial fluid, tears, mouse serum, etc. Recent improvements include the new MAGPIX system and the introduction of magnetic MagPlex microspheres, (Bio-Plex Pro™ magnetic COOH beads) which have reduced the cost of performing a singleplex assay, as expensive filter plates are no longer required.

The Thomas Joos Laboratory at the Natural and Medical Sciences Institute (NMI) at the University of Tübingen performed a comparison of a sandwich immunoassay on ELISA and xMAP Technology using the Bio-Plex® MAGPIX™ multiplex reader. The assay was built to detect the soluble form of the tumor necrosis factor receptor 2 (TNFR2). Parallel assays were built using the same antibodies and recombinant standard protein. The same material preparations (for example, serial dilutions of standard proteins) were utilized to achieve the best possible comparison.

Reduce Costs and Total Labor Time Using the Bio-Plex MAGPIX Multiplex Reader

The costs for the bead-based sandwich immunoassay and ELISA were calculated based on experiments performed at NMI. The calculations are based on the exact requirements for a 96-well plate. Actual costs could be slightly higher due to materials such as standards that could be used for a large number of assays, but might have to be disposed of due to shelf life, or thawed aliquots that have to be discarded due to shelf life issues. Both costs were calculated using a seven-point standard curve and assuming that there were 80 samples per plate and 16 wells used for background and standards. The costs were based in Euros and the Bio-Plex MAGPIX assay was less than half the cost of the standard ELISA, utilizing the same pair of antibodies and the same recombinant standard.

Table 1. Cost comparison of xMAP assay on Bio-Plex MAGPIX reader versus an ELISA assay*.

Bio-Plex MAGPIX Reader

Item	Price	Price/Plate (96 wells)
ELISA microplate	€0.97 per plate	€0.97
MagPlex beads	€469.00 per ml	€11.20
Capture antibody	€330.00 per 500 µg	€0.70
Standard protein	€345.00 per 50 µg	€0.12
Detection antibody	€425.00 per 50 µg	€7.40
Goat serum	€49.00 per 25 ml	€0.12
Streptavidin-PE	€250.00 per 1 ml	€1.75
Microplate assay sealing film	€49.00 per 100 pieces	€0.49
Buffers, etc.		€1.00
	TOTAL	€23.75

ELISA

Item	Price	Price/Plate (96 wells)
ELISA microplate	€0.97 per plate	€0.97
Capture antibody	€330.00 per 500 µg	€12.67
Standard protein	€345.00 per 50 µg	€0.12
Detection antibody	€425.00 per 50 µg	€24.48
Goat serum	€49.00 per 25 ml	€0.38
Streptavidin-HRP	€250.00 per 1 ml	€12.00
TMB-substrate	€49.00 per 100 ml	€4.70
Microplate assay sealing film	€49.00 per 100 pieces	€0.49
Stop solution (1 M HCl)	€13.55 per 1L	€0.19
	TOTAL	€56.00

* Costs were calculated and reported based on experiments performed by the Thomas Joos Laboratory at Natural and Medical Sciences Institute (NMI) at the University of Tübingen. Costs are shown for illustrative purposes only.

In addition to the assay cost comparison, the investment in capital equipment required to run these assays should be considered. With the recent launch of the new Bio-Plex MAGPIX reader, access to xMAP technology and the benefits of multiplexing has now become more affordable. 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.

The total assay time and hands-on time were measured for both assay formats. Time for couple detection antibodies to beads and coating of ELISA plates was not added, as this is done for many assays at once. The total assay time is similar for both assay formats, although the ELISA has more steps. This is because the Bio-Plex MAGPIX reader has a longer read time than an absorbance plate reader. Labor costs will be similar for the two assays when comparing a singleplex assay. If multiple analytes are being examined, then labor costs would increase for the ELISA, but remain the same for the Bio-Plex MAGPIX reader. Magnetic beads allow for automation of workflow and better reproducibility compared to the previous generation of polystyrene beads.

Analytical Performance of xMAP Technology is Comparable to ELISA

The range of both assays was determined using a threefold serial dilution of the standard recombinant protein, starting at 10,000 pg/ml with a total of seven standard concentrations. Both assays performed over the range of 14–10,000 pg/ml, with the Bio-Plex MAGPIX assay having a more linear range than the ELISA (Figure 1).

Table 2. Time comparison of ELISA and xMAP assays.

Bio-Plex MAGPIX Reader

	Assay Time, min	Hands-on Time, min
Blocking of (ELISA) microtiter plate*	30	5
Dilution of standard protein and preparation of samples (1 standard curve in duplicate and 80 samples)	40	40
Pipetting of 100 µl sample or standard to each well	10	10
Incubation	120	
Washing step (3 x 100 µl) using magnetic plate	5	5
Preparation of detection antibody solution	2	2
Adding 30 µl of the detection antibody solution per well	3	3
Incubation with detection antibody	60	
Washing step (3 x 100 µl) using magnetic plate	5	5
Prepartion of streptavidin-phycoerythrin solution	2	2
Adding 30 µl streptavidin-PE per well	3	3
Incubation	30	
Washing step (3 x 100 µl) using magnetic plate	5	5
Assay read-out	80	5
Total assay time	**6 hr 30 min**	
Total hands-on time		**1 hr 35 min**

* Bio-Plex assays do not require a blocking step.

ELISA

	Assay Time, min	Hands-on Time, min
Dilution of standard protein and preparation of samples (1 standard curve in duplicate and 80 samples)	40	40
Pipetting of 100 µl sample or standard to each well	10	10
Mixing	1	1
Incubation	120	
Washing step (3 x 400 µl)	5	5
Preparation of detection antibody solution	2	2
Adding 100 µl of the detection antibody solution per well	3	3
Incubation at room temperature	120	
Washing step (3 x 400 µl)	5	5
Preparation of streptavidin-HRP solution	2	2
Adding 100 µl streptavidin-HRP per well	3	3
Incubation	20	
Washing step (3 x 400 µl)	5	5
Dilution of substrate solution	1	1
Adding 100 µl substrate solution per well	2	2
Incubation	30	
Adding 50 µl stop solution per well	2	2
Assay read-out	10	5
Total assay time	**6 hr 20 min**	
Total hands-on time		**1 hr 40 min**

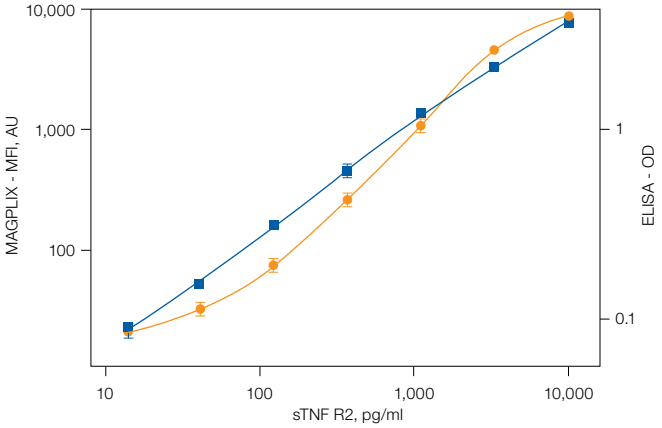

Fig. 1. Standard curves of ELISA (●) and xMAP assay performed on Bio-Plex MAGPIX (■).

The limit of detection (LOD) of the TNFR2 assay was 4 pg/ml and the limit of quantitation (LOQ) was 10 pg/ml. For the ELISA, the LOD was 9 pg/ml and the LOQ was calculated to be 50 pg/ml. LOD was defined as the recalculated concentration obtained by adding three standard deviations to the background reading. LOQ was calculated using the same process, but with the addition of ten standard deviations to background. The Bio-Plex MAGPIX assay had a twofold more sensitive LOD and was fivefold more sensitive for LOQ. The LOD for each assay is dependent upon the proper selection of antibody pairs and standards. Assays can reach detection limits of less than 1 pg/ml.

Analytical comparison of the two assay formats demonstrates that the bead-based xMAP assay can be used to obtain comparable results with improved efficiency and performance over the traditional ELISA format. Historical data obtained using ELISA can be maintained while transitioning to xMAP assays on the Bio-Plex MAGPIX reader, enabling researchers to continue long-standing studies and access the added benefits of xMAP technology. In addition to the benefits demonstrated by a singleplex assay format, conversion to xMAP technology provides researchers the added benefit of multiplexing several tests in the same well, further increasing efficiency and reducing time and costs in the laboratory.

Publications Support Findings in a Multiplex Format

In addition to the recent study outlined above, there have been numerous laboratories that have evaluated the performance of xMAP technology over the years (de Jager et al. 2003, de Jager et al. 2005, duPont et al. 2005, Codorean et al. 2010, Richens et al. 2010). Coincident with the NMI study, a group at Bristol-Myers Squibb (BMS) was pursuing development of a more sensitive assay for IL-23 than they could obtain commercially (Rizzi et al. 2010). They utilized the same pair of antibodies as available in one of the two commercial ELISAs they evaluated. The xMAP assay provided the best precision and accuracy, had the widest range of detection, and the highest sensitivity compared to the two commercial kits. Rizzi et al. also combined the singleplex IL-23 assay with other biomarkers and found that it performed well in either a singleplex or multiplex format. In the singleplex format the xMAP assay had excellent correlation to the two ELISAs (r^2 ~0.9). In the past only multiplex studies had been performed comparing xMAP to ELISA. The BMS group provided enough information in the paper to do a cost analysis similar to the NMI group. The cost of the xMAP assay was less than one-fifth of the less expensive commercial ELISA they used and less than one-sixteenth of the more expensive commercial kit.

The explanation of why the xMAP-based assays perform better and are less expensive than ELISA is based on several factors. The first is that the xMAP assays are based on direct fluorescence detection as opposed to colorimetric detection mediated by an enzyme, resulting in better sensitivity. Second, capture antibodies have higher avidity and lower background due to covalent coupling to beads, 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. The higher background of ELISA plates is due to the fact that they have a much larger surface area than the combined area of 2,500 beads, and are therefore more prone to nonspecific binding of detection antibody (Carson and Vignali 2000). If blocking of the large surface area of the microplate is not performed correctly, any proteins can bind. Finally, cost is reduced because there is less capture antibody used due to the smaller surface area.

Conclusion

xMAP technology offers 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. Both Rizzi et al. and NMI demonstrated that xMAP assays using the same antibody pairs out-perform ELISA and show significant cost savings based on the cost of materials, notably antibodies, used in the assay. Labor should be equal in a singleplex comparison, as the assay protocols are approximately the same length. However, xMAP technology offers the flexibility to multiplex as well, and in that case labor cost would decrease by a factor of the multiplex level. That is, labor costs would decrease by one-half if a 2-plex were performed.

The Bio-Plex MAGPIX system using xMAP technology offers significant advantages over ELISA, especially material savings that can be very substantial when compared to commercial ELISA kits. Researchers do not have to multiplex to save money by moving to the xMAP format. However, there is the option of multiplexing, which can save time and sample, proportional to the number of analytes.

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