Application Note

Automated Workflows for Luminex xMAP® Assays

Paul Held and Peter Banks, BioTek Instruments, Inc., Winooski, Vermont

Bead-based, multiplexing assays such as those provided by the Luminex xMAP® technology, have proven to be highly useful for biomarker identification and quantification from cells, tissues and body fluids. Applications include biological research, drug discovery and clinical diagnostics. The original technology based on the use of polystyrene microspheres tends to have arduous, manual work flows prone to cumulative systematic and random error that can lead to problematic precision for quantification. Here we demonstrate improvements in precision and ease-of-use through the use of BioTek microplate washers for both polystyrene and magnetic microspheres. Automation of workflows for partially-full microplates to full 96- and 384-well microplates can be performed.

Introduction

The use of biomarkers by practitioners to detect disease and improve therapy is an old practice. Pulse and blood pressure measurements are general methods for most physical examination – these are biomarkers of general health that have a long history. Today, biomarkers are often molecular in nature. Often improved relevance to the disease or drug effect can be obtained through the use of multiple biomarkers. The Luminex xMAP technology is one of the most commonly used multiplexing technologies for the simultaneous measurement of multiple biomarkers. There are currently 37 Luminex partners that manufacture reagent systems based on xMAP technology for research, drug discovery and clinical diagnostic applications[1].

The original xMAP technology is based on the use of polystyrene microspheres. Microplate-based sample preparation involved the use of vacuum filtration to remove sample matrix and excess reagents in a fashion similar to cell membrane-based ligand receptor binding experiments. While acceptable analytical performance can be achieved, the process is prone to individual clogged wells and is difficult to fully automate. Magnetic microspheres have recently become available that have significantly improved ease of use and make the xMAP technology much more automation friendly. In this application note, we will demonstrate performance attributes of a series of BioTek microplate washers for the automation of xMAP assays: from a strip washer for polystyrene microsperes to full microplate washing using magnetic microspheres.

BioTek Microplate Washers

The BioTek product line of microplate washers suitable for xMAP assays consists of the ELx50™ Strip Washer, ELx405™ Full Microplate Washer and the EL406™ Combination Washer Dispenser. The full plate washers are available with BioTek's Dual Action Manifold™, providing independent control of both dispense and aspirate manifolds for efficient bead washing and low residual volumes. The products also use angled dispense tubes, full control of dispense and aspiration rates and unique X, Y, Z positioning to ensure good bead recovery, whatever the microplate density or conformation. Each product has models equipped with vacuum filtration capability for use with polystyrene beads; or models with a high field strength magnet appropriate for magnetic beads. The ELx50 Strip Washer is suitable for low throughput operation; ELx405 is better suited to higher throughput applications and EL406 combines ELx405 washing capability with peristaltic pump-based and/or syringe pump-based reagent addition.

Figure 1. Product line of BioTek washer products suitable for Luminex xMAP assays: Left: ELx50 model with vacuum filtration capability suitable for polystyrene microspheres (magnetic microsphere models also available); Center: ELx405 model with high field strength magnet for magnetic microspheres (vacuum filtration models also available); Right: EL406 model with high field strength magnet for magnetic microspheres and reagent dispensing applications (vacuum filtration models also available).

Typical Automated Work Flows for Quantitative Assays

Protein Biomarkers

The work flow for xMAP® protein biomarker samples is essentially the same for polystyrene or magnetic microspheres. Typically the work flows use conventional ELISA-based strategies of immobilization of analyte using a primary antibody to a support (i.e. microsphere), followed by the addition of a secondary tagged-antibody to a different epitope on the protein and ending with a detection reagent which will bind to the tag on the secondary antibody. Wash steps are either required after introduction of the sample if complex sample matrices are used (to reduce background signal) or after the final addition of detection reagent to simplify work flow. The work flow is presented in Figure 2 below.

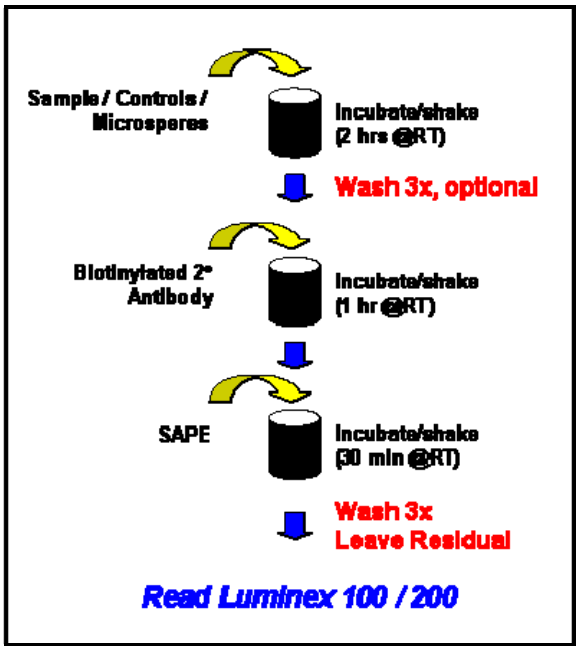

Figure 2. Typical work flow for protein biomarker quantification. Red text indicates use of BioTek washers to automate the process. SAPE refers to streptavidin-conjugated phycoerythrin as a detection reagent.

Gene Expression Biomarkers

Gene expression biomarker quantification typically benefits from an amplification process. Either this can take to form of first converting mRNA into DNA using reverse transcriptase enzymes and then amplifying the product using conventional polymerase chain reaction (PCR) technology or the binding reagent to the mRNA can be amplified using branched DNA technology. This is the basis of the QuantiGene technology commercialized by Panomics (now Affymetrix) and depicted in figure 3 below:

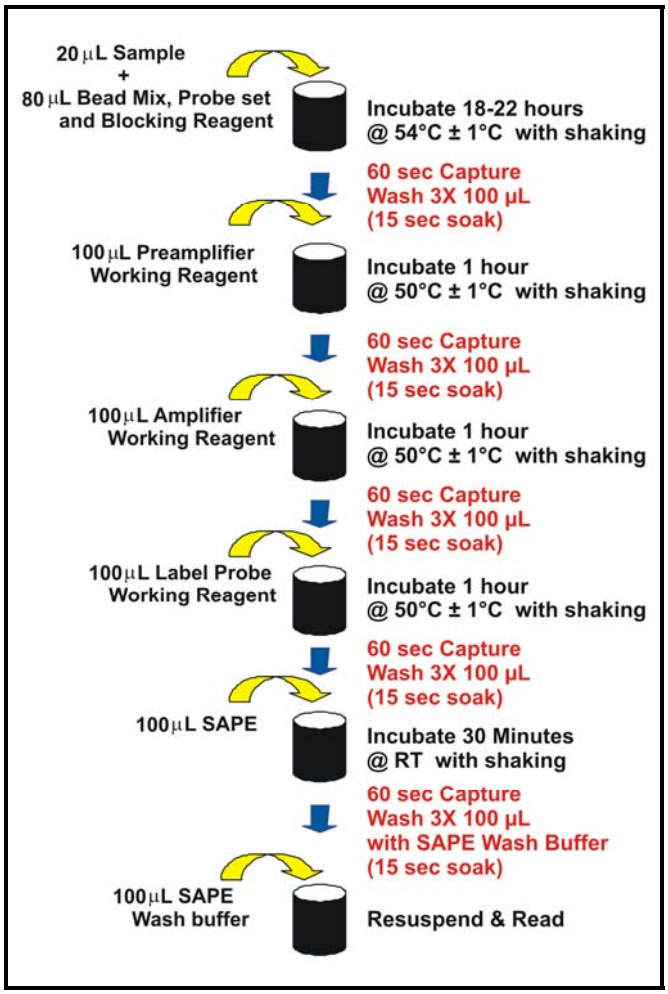

Figure 3. Typical work flow for gene expression biomarker quantification. Red text indicates use of BioTek washers to automate the process. SAPE refers to streptavidin-conjugated phycoerythrin as a detection reagent.

It is obvious from a comparison of work flows that the automation of gene expression biomarker quantification is more complicated and prone to more random error from the multiple steps involved in sample processing relative to protein biomarkers.

Methods

Assays were performed for protein biomarker quantification using both polystyrene microspheres and vacuum filtration provided by ELx50™ and magnetic microspheres using ELx405™ supplied with a high field strength magnet. Gene expression biomarker quantification was performed with magnetic microspheres and the same ELx405 model as described above. A full description of instrument settings and materials are available elsewhere[2-4].

Results and Discussion

Protein Biomarkers – Vacuum Filtration with ELx50™

Automation of vacuum filtration serves to provide greater "ease-of-use" than manually using separate vacuum manifolds and processes rows of samples and full microplates much more quickly. Below in Figure 4, a single plex thyroid stimulating hormone (TSH) xMAP® assay was conducted with standard amounts of TSH. Each standard was measured in replicate from 24 wells and median fluorescent intensities (MFI) measured with a Luminex 200 system. Comparisons were made between automated workflows using ELx50 (blue bars) and manually using a widely available vacuum manifold (red bars).

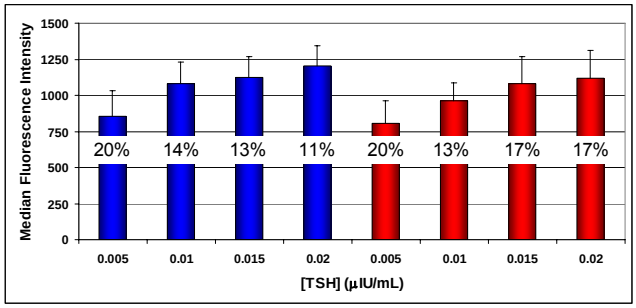

Figure 4. Median fluorescence intensities averaged from 24 replicates of four standard amounts of TSH. Blue bars represent ELx50 automated sample processing; red bars represent manual processing. Error bars represent 1 standard deviation in the replicate measurements. Percentages embedded in the columns represent %CV in the replicate measurements.

It is evident that MFI for the ELx50 processed samples was slightly higher indicative of improved bead recovery from the wash process. Also, in general, improved precision is noted, by lower CVs in the higher TSH concentrations.

Protein Biomarkers – Magnetic Washing with ELx405™

The ELx405 is a full plate washer that uses a custom neodymium iron boron magnet to perform magnet-based washing in both 96- and 384-well microplates. Full plate washing provides very rapid sample processing with excellent washing efficiency and bead recovery. Figure 5 demonstrates calibration curves for a 13-plex human metabolic hormone panel. The data demonstrates that using the ELx405 to wash magnetic microspere-based multiplex assays in a 96-well microplate format results in very reliable data. These standard curves can be used to calculate unknown sample concentrations with a high degree of confidence.

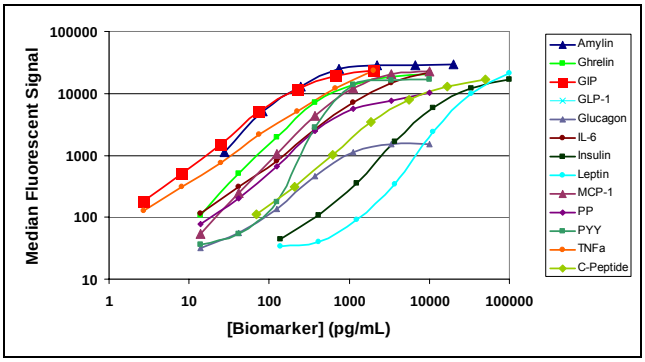

Figure 5. Calibration curves for the 13-plex human metabolic hormone panel which uses magnetic microspheres.

Equally important is the degree of precision obtained in the analysis. As demonstrated in Figure 6, comparisons were made with using magnetic microspheres in conjunction with the ELx405 Magnetic Bead Washer, a commercially-available magnetic strip washer and standard filter washing via a vacuum filtration manifold using the same beads. While the standard curves were all essentially the same (data not shown), the precision of the replicate data for each of the three sample processing methods was analyzed in a histogram, it was noted that the ELx405 produced significantly higher precision and was not prone to high variance data indicative of loss of beads by filter plate clogging.

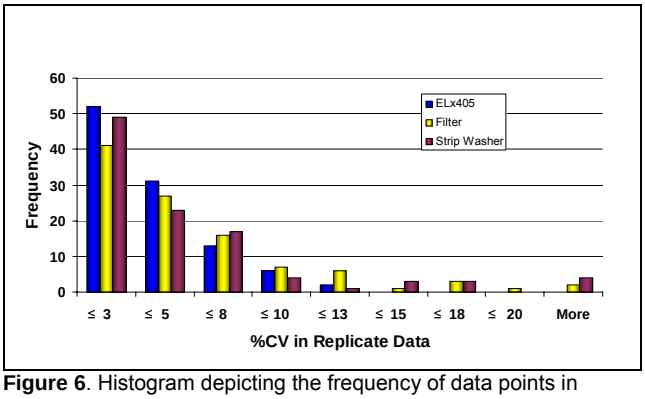

Figure 6. Histogram depicting the frequency of data points in Figure 5 with CVs depicted on the abscissa (x-axis).

Gene Expression Biomarkers – Magnetic Washing with ELx405

As noted in the biomarker workflows, sample processing for gene expression biomarker xMAP assays has appreciably more steps relative to protein biomarkers and automation of the workflow can have significant advantages relative to manual processing. Removal of unbound materials is a critical component to providing accurate and reliable data. In regards to the use of an automated washer such as the ELx405, well-to-well uniformity depends on accurate and repeatable dispense-volumes, low residual volumes and reliable bead retention during the wash steps. By using a 36-plex assay of house-keeping genes in conjunction with purified known RNA samples, the uniformity of signal response when using the ELx405 Magnetic Bead Washer to wash the assay in 96-well microplates can be examined. As demonstrated in Figure 7, the fluorescent signal of an RNA species is consistent across the entire plate.

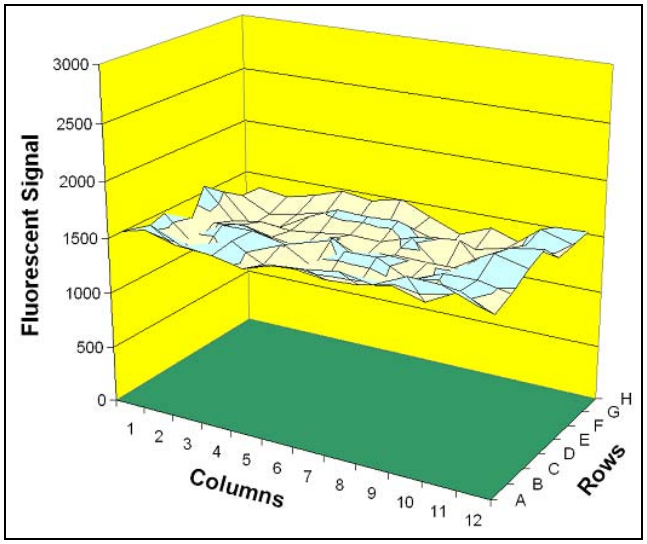

Figure 7. Surface plot demonstrating well-to-well Signal Uniformity. NFKB1 RNA was measured from purified RNA aliquoted into all of the wells of a 96-well microplate as part of a 36-plex QuantiGene Plex assay. The fluorescent signal was then plotted using Microsoft Excel.

A difficult challenge is to perform wash steps in 384-well microplates. The demand to reduce sample size in order to decrease reagent usage has provided the impetus to higher density plate formats. However, the smaller well size makes the aspiration of fluids more difficult in regards to bead retention. By increasing the height of the aspiration tubes relative the bottom of the well, adequate washing was obtained without significant loss of the beads.

Figure 8 demonstrates the ability of the ELx405™ to wash magnetic beads in a 384-well format. Comparing a sample of purified total RNA isolated from liver to the Universal Reference RNA (URR) four different samples were taken from each pool and assayed in 4 replicates each. Despite the difficulty in washing and retaining beads, the blank wells remained low, while the experimental wells had orders of magnitude greater signal. In addition, replicate samples had very similar results.

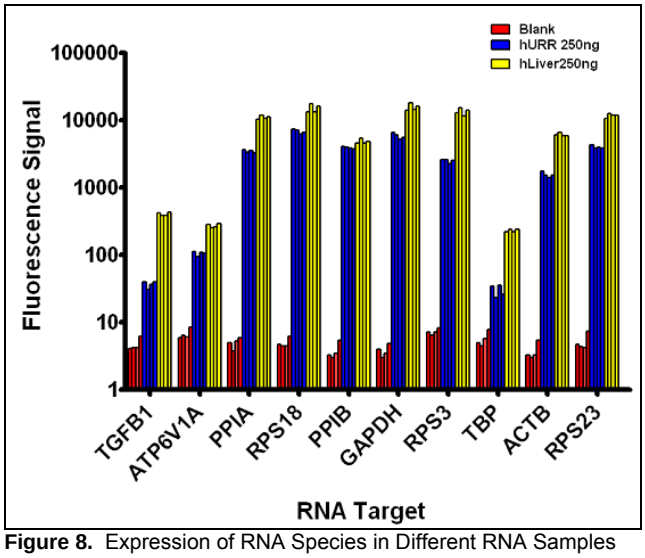

Figure 8. Expression of RNA Species in Different RNA Samples using 384-well Reaction Plates.

Conclusions

We have demonstrated the ability of BioTek microplate washers to automate the wash processing needed for xMAP® biomarker quantification assays, whether they are protein or gene expression biomarkers and whether the assays are based on polystyrene or magnetic microspheres. Automation serves to simplify and speed processing, but it also tends to improve bead recovery to increase MFI, improve washing efficiency to reduce background and improve precision leading to better assay performance. The three different models of BioTek washer provide the end-user with three different levels of automation: strip washing with ELx50™ for lower throughput; full plate washing with ELx405 for higher throughput; and EL406™, which has all the attributes of ELx405 for washing applications, but also the ability to dispense xMAP® reagents for more comprehensive automation of sample processing.

References

1. http://www.luminexcorp.com/partners/index.html
2. Held, Paul. "Using the ELx50 Filter Microplate Washer to Perform the Wash Processing Steps Required for Luminex xMAP Assays." Application note available at www.biotek.com, April, 12, 2007.
3. Held, Paul. Automated Magnetic Bead Washing of Metabolic Hormone Assays." Application note available at www.biotek.com, Sept, 18, 2009.
4. Held, Paul; Nguyen, Nina; Tran, Bao-Huy; and Lu, Binh. "Automated Washing of Magnetic Bead QuantiGene Plex 2.0 Assays." Application note available at www.biotek.com, Sept, 16, 2009.

Rev. 11/05/09

RA44420.4998148148

5994-2625EN
September 1, 2021