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Abstract

Luminex® xMAP® technology, utilizing microspheres as a solid support, continues to grow in its application for immunological research and diagnostics. This technology is frequently used, for instance, in serological assays to assess the presence of autoimmune disease or other immune responses. Increased utilization of this technology is driving the need for scale-up and improved consistency through automation.

We demonstrate the utility of an automation compatible filter plate to enable easy transfer of protocols to common liquid handling platforms. Using rat and mouse cytokine panels as a model system we demonstrate compatibility between filter plates offered in a common kit (Millipore Linco, St. Louis, MO) and a high throughput compatible filter plate available from Millipore specifically designed for use with automated equipment. The plate dimensions are standardized to meet ANSI/SBS 2004 for multiwell plates and are fully compatible with automated gripper arms, stackers and plate readers. Rigid sidewalls also provide ample surfaces for bar codes. All of these features are important when considering scale up of any assay. Data generated both manually and using automation will be presented demonstrating the simplicity of automating and scaling up assays using Luminex technologies.

Introduction

LINCO*plex* assay kits for Mouse and Rat Cytokines are used for the multiplex analysis of soluble immune mediators in serum, plasma, tissue extracts, or other biological fluids. The use of microspheres with two fluorophores (Luminex xMAP technology) allows the detection of multiple analytes in a single reaction. Desired molecules or analytes are bound to the microspheres (or beads) and then tagged with an fluorescent reporter antibody. The microspheres are read using a dual laser Luminex 100 IS analyzer to generate the median fluorescent intensity (MFI) signal strength of these multiple analytes. This dual laser system allows the detection of the microspheres with one laser, and the detection of the specific fluorescently tagged samples with the other laser.

In the past, these assays had been run in tubes (one tube per sample) which resulted in lost beads and samples during the multiple wash steps and transfer steps. The LINCO*plex* kits shown here utilize a 96 well filter plate. The filter plate eliminates the need for multiple transfer steps for rapid, uniform washing to remove excess reagents and prevent microsphere loss.

Another advantage of using filter plates in these assays is the ability to automate the process. The filter plate used in the kits (MultiScreen®$_{HTS}$ filter plate) can be handled by robotic systems since it is designed with rigid walls (which grippers can move) and meet the ANSI/SBS 2004 1-4 standard compliance. The Tecan Freedom EVO® workstation (shown here) with an integrated vacuum station, RoMa arm and fixed tips allows automation of both the Mouse and Rat Cytokines LINCO*plex* assay kits.

Automation Workstation

A.

B.

Figure 1 (A & B). The Tecan Freedom EVO workstation (1A) with 8 channels allows for direct pipetting from reservoirs as well as 1.5 ml centrifuge tubes into a MultiScreen$_{HTS}$ filter plate. The on deck vacuum option with a MultiScreen$_{HTS}$ vacuum manifold and the RoMa arm allows total automation of all the filtration steps. The Mécour™ cooling/heating block (1B) with a VARIOMAG™ shaker was used for all automated incubation steps.

Methods

Pre-wet filter plate

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Add Assay Buffer, Standards, Cytokine Controls (QC-1, QC-2) & Serum Matrix

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Add Premixed Multiplexed Microspheres and incubate overnight, 4 °C

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Wash beads two times, add Detection Antibody Cocktail, incubate

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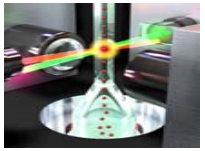
Add Streptavidin-Phycoerythrin, incubate, followed by washing

↓

Resuspend in Sheath Fluid & read on the Luminex 100 IS analyzer



Mouse Cytokine Assay: Automation vs. Manual

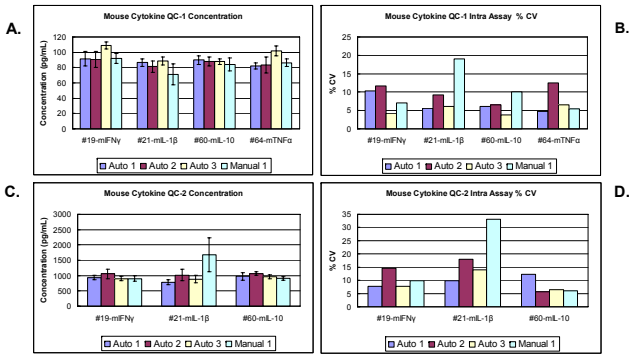


Figure 2 (A - D). QC-1 and QC-2 control concentrations (2A & 2C) were determined for each of the analytes tested on separate days of automation (Auto 1, 2, 3) vs. a manual plate (Manual 1). The intra assay bead (same type of beads in well) % CV (2B & 2D) was determined for each plate run for QC-1 and QC-2. Automation samples tended to have lower standard deviations compared to the manual plate for each analyte. The QC-2 #64-mTNFα data is not shown (beyond linear range of standard curves). The % CV for automation was typically ≤ 15% for most analytes tested. Data was calculated using a curve fitting software (BeadView™ Multiplex Data Analysis Software) with a 5 parameter logistic method.

Rat Cytokine Assay: Automation vs. Manual

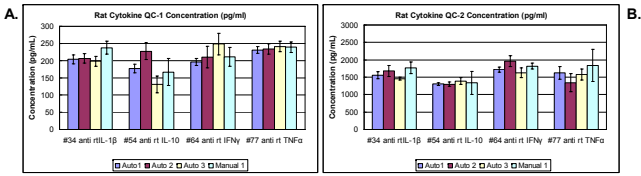


Figure 3 (A - B). QC-1 and QC-2 control concentrations (3A & 3B) were determined for each of the four analytes tested on separate days of automation vs. a manual plate. The intra assay bead % CV was determined for each plate run for QC-1 & QC-2 (data not shown). As with the Mouse kits, the % CV for automation was typically ≤ 15% for most analytes tested. The data was calculated using a curve fitting software (BeadView Multiplex Data Analysis Software) with a 5 parameter logistic method.

Rat Cytokine Standard Curves: Automation vs. Manual

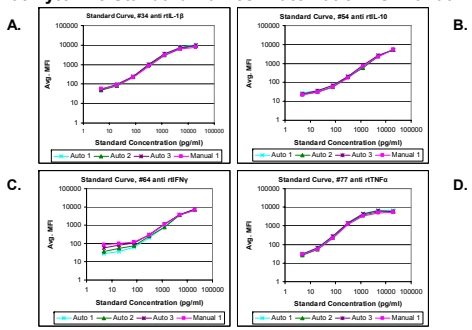


Figure 4 (A - D). The MFI for the standard curves (4A - 4D) were determined for each of the four analytes tested on various days of automation vs. a manual plate. The QC-1 and QC-2 samples were calculated within the linear portion of the standard curves. Both the automated and manual testing generated similar curves for the Rat Cytokine and Mouse Cytokine (data not shown) kits.

Conclusions

- LINCO*plex* Mouse and Rat Cytokine kits can be automated to meet the needs of assay scale-up.
  - ✓Full automation including standards
  - ✓Microsphere washing/filtration protocols
  - ✓Temperature control for filter plate incubation using Mécour cooling/heating block
- MultiScreen$_{HTS}$ filter plate design is compatible with most automation systems, such as the Tecan Freedom EVO 8 tip workstation.
- Automation in a complex assay system:
  - ✓Reduces hands-on time
  - ✓Minimizes pipetting errors
  - ✓Improves consistency, data reliability, and lowers standard deviation
- No significant assay performance differences between automated and manually run samples.
- Automation Intra Assay % CV ranges are within an acceptable range for assay.