U.S. Department of Health and Human Services
National Institutes of Health
NIH National Heart, Lung, and Blood Institute

Novel Approach to Quantify Soluble Proteins by Multiplex Bead Arrays Minimizes Sample and Reagent Requirements

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INTRODUCTION

The measurement of soluble proteins from bodily fluids and tissue culture supernatants is widely used to study inflammation, immune responses, metabolic regulators, and pathways associated with disease. Commonly used technologies for soluble protein detection, such as Luminex bead array assays, have the benefit of allowing the multi-plexing of up to approximately 40 analytes in one reaction, drastically minimizing the amount of sample required. None the less, costs increase as the number of analytes assayed increases, somewhat limiting their appeal. Additionally, there has been a push in the field to reduce the amount of sample volume requirement, minimizing the need to draw large volumes of blood from patients, and is especially useful when sample is extremely limited.

Curiox has developed a novel technology, Drop Array, to minimize both the amount of sample and reagent required for conventional Luminex assays, while maintaining the ability to use the kits and instrumentation labs already have in place. The technology centers on the Drop Array plate, which contains 96 hydrophilic resin coated wall-less wells on a hydrophobic resin coated plate. The "wells" allow for approximately 1/5th to 1/10th the amount of sample and beads required for conventional Luminex, with a maximum volume of 20ul. Crucial to the Drop Array technology are the anti-evaporation lid, humidified box, and LT-MX magnetic based plate washer. Another aspect that distinguishes the Drop Array technology from conventional Luminex assays is that the incubation with antibody coupled beads occurs on a magnet, resulting in an even monolayer of beads (as does the plate washing), increasing the binding (and washing) surface area, according to Curiox.

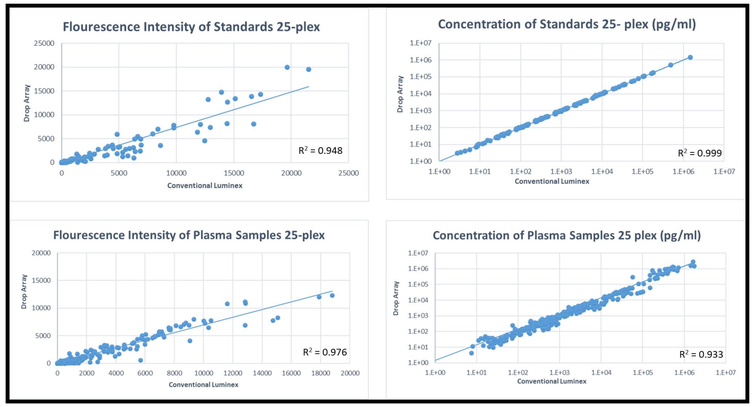
Depiction of Drop Array microplate and LT-210 magnetic washer. Images provided by Curiox.

METHODS

- **Concentrations of soluble proteins in human plasma or cell culture supernatants were measured by conventional Luminex assays and by Drop Array technology. All assays were run on a Luminex 100. Data were acquired and analyzed using Bio-Plex Manager 6.1 software. Wells were designated as outliers if they met any of the following criteria:**
  - **Fluorescence Intensity was below the lower limit of detection (LLOD = {2 x standard deviation of background fluorescence intensity} + mean of background fluorescence intensity)**
  - **Percent recovery ({observed concentration/expected concentration} x 100) was <70% or > 130%**
  - **Bead count was < 30**
- **35 human plasma samples were assayed by an R&D Screening Assay 25-Plex containing MCP-1, MIP-1a, DC40 Ligand, MIG, G-CSF, IFNg, IL-10, IL-17A, IL-2, IL-5, MMP-1, IL-1b, MMP-7, MDC, MIP-1b, IP-10, E-Selectin, ICAM-1, IL-1a, IL-12p70, IL-1ra, IL-4, Leptin, VEGF, and MCP-3 at a 1:2 dilution.**
- **40 cell culture supernatants were assayed by an R&D Screening Assay 17-Plex containing TNFa, IL-8, IL-10, GRO beta, PARC, MIP-1a, MIP-1b, IL-12p70, IL-23, IL-6, IP-10, MCP-1, CXCL13, Eotaxin, MDC, MIG, and TARC at a 1:2 dilution.**
- **24 cell culture supernatants were assayed by an R&D Screening Assay 10-Plex containing Endothelin-1, FGF basic, MMP-1, MMP-9, PIGF, FGF acidic, HB-EGF, MMP-3, PDGF-BB, and VEGF at a 1:2 dilution.**
- **Conventional assays were run per manufacturer instructions. Drop Array assays had the following changes:**
  - **5ul volumes added onto Drop Array plate at all steps except final**
  - **Drop Array Plate was vortexed before incubations**
  - **Pipetting and incubation steps occurred on a magnet with the plate under the humidified lid in the humidified box**
  - **10ul wash buffer added to plate after final wash. Reading grid is placed on top of plate to create deeper wells, and volume is brought up to 65ul total.**
  - **Probe height on Luminex instrument was adjusted. 50ul volume was read on Luminex 100.**

RESULTS

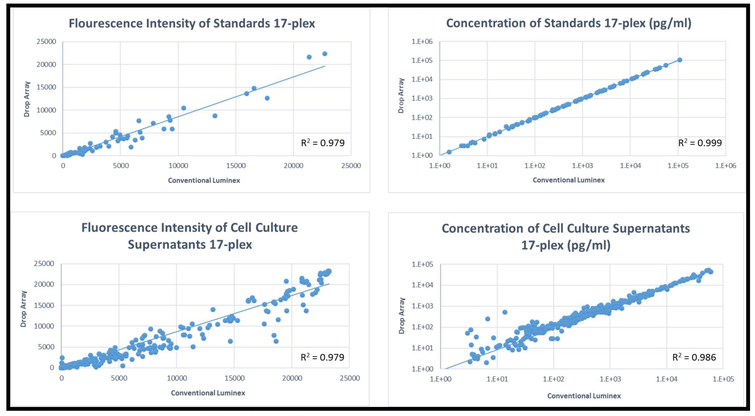
A) Correlation of Conventional Luminex and Drop Array on 25-plex with human plasma samples



Correlation of fluorescence intensities and concentrations obtained from conventional Luminex and Drop Array. When one platform resulted in a concentration in range while the other platform resulted in a value above or below the level of quantification for a given analyte, the data point was assigned the upper limit of quantification concentration value or 0.001, respectively. Where data points from both platforms were above upper or below lower limit of quantification, data points were omitted.

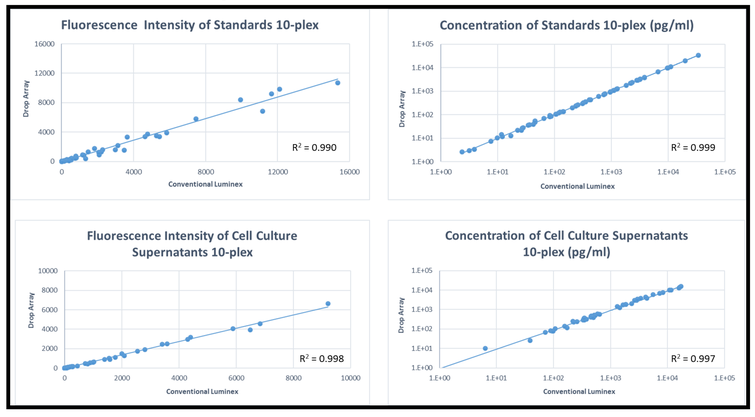
RESULTS

B) Correlation of Conventional Luminex and Drop Array on 17-plex with human cell culture supernatants



***MDC data has been omitted from this analysis as numerous points on the Drop Array standard curve failed to meet our criteria (see methods), which resulted in loss of an acceptable standard curve.**

C) Correlation of Conventional Luminex and Drop Array on 10-plex with human cell culture supernatants



DISCUSSION

Drop Array technology significantly increases the number of samples possible to assay with one kit, and decreases the required sample volume. We have found that data from the Drop Array platform are comparable to conventional Luminex. Care must be taken to ensure proper probe height, proper mixing of samples, and optimal operational of Drop Array plate washer. The data presented here experienced one or all of the aforementioned issues, which may have contributed to lower bead counts and lower fluorescence intensities on Drop Array than Luminex. Nevertheless, there was good correlation of concentrations, which seemed to decrease with increasing number of analytes. Correlation of fluorescence intensity was lower than that of concentration. Correlation of standard concentrations was higher than of samples, due to differences in complexities of recombinant protein and biological samples.