utilisation of a novel independent multi-channel dispenser for rapid assay development of a bead based no-wash sandwich ELISA assay

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Introduction

Every assay, whether for screening, hit to lead or lead optimisation, requires significant experimental optimisation for robust assay performance. Variables requiring optimisation can include things like antibody concentrations, bead number, buffer constituents, pH, as well as a variety of incubation time points. However, with a manual approach the number of parameters that can be varied is often limited due to the sheer difficulty and time it would take to dispense all the components in the desired combinations, leading to long and iterative assay development cycles.

Here we have used a novel positive displacement dispensing instrument to reduce assay development times to develop no wash, bead-based assays from commercially available ELISA kits. Parameters such as different buffer components, concentrations of detection antibodies and fluorescent labels have been investigated for optimal assay performance.

In this poster, we demonstrate a no-wash 5-plex cytokine assay in a 384 well plate that has been converted from a standard ELISA plate based format by using the dragonfly® discovery automated pipettor.

1. dispense technology

non-contact dispensing from a disposable, positive displacement tip

In each of the channels (up to 10) there is a tight fitting piston that travels within a pipette barrel, when coupled to the instruments piston rod the positive displacement syringe is formed. The distance and rates of acceleration and deceleration of the piston control how and when liquid is ejected from the tip.

Each channel is fully independent of the others, yet they can all be operated simultaneously, giving rapid, but highly flexible dispensing. This enables complex combination gradients to be set up in high density (up to 1,536-well) microplates, as well as high speed bulk filling of common reagents.

Fig 1 dragonfly discovery

2. conversion of ELISA to bead assay

Traditional colourimetric ELISA assays require multiple wash steps and only allow analysis for a single target per well. The sol-R™ bead-based assay format facilitates multiplexed no wash fluorescent ELISA assays.

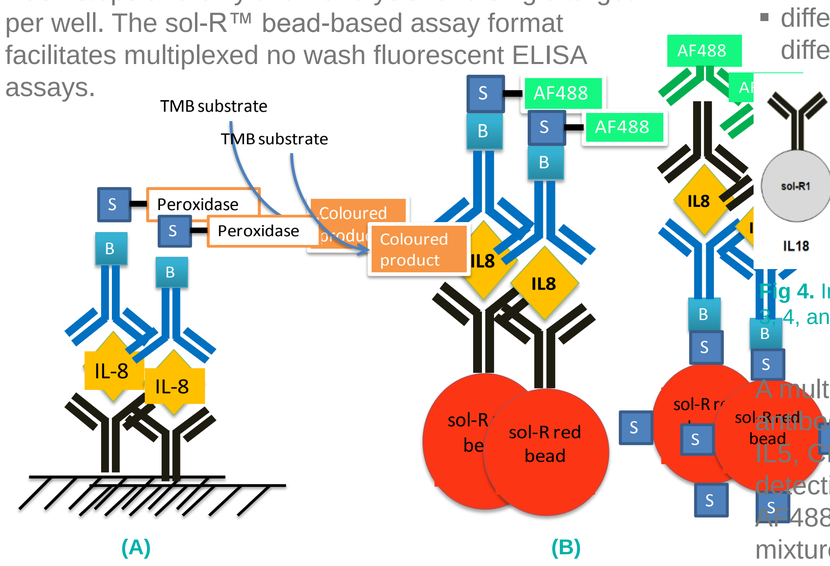


Fig 2. Comparison of ELISA (A) and sol-R bead (B) assay binding schematics. When converting ELISA onto sol-R bead assay format, the capture antibody is immobilised onto the bead. The same biotinylated detection antibody is used in both assay formats but the detection system is changed to fluorescent Alexa Fluor 488 – conjugated streptavidin for the bead assay.

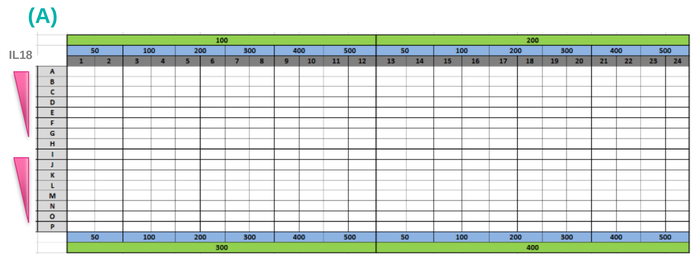
Bead-based assays offer several advantages:

- compatibility with 96, 384 or 1,536 well plates
- specialised mirrorball ® reader system rejects background fluorescence, enabling a no wash assay format
- coded beads enable multiplex assays

3. use of a novel multi-channel dispenser for bead assay optimisation

dragonfly discovery was used to optimise the relative concentrations of biotinylated detection antibody and AF488-streptavidin in a single plex bead based assay quantifying IL18 cytokine in 384 well plate in a total assay well volume of 20µL (figure 3). Twenty-four different combinations of biotinylated detection antibody (shown in blue) and AF488-streptavidin (shown in green) concentration were tested against an 8-point dilution series of IL18 cytokine, allowing identification of conditions giving best results(Fig 3B, highlighted in red) (highlighted in red)

(A)



(B)

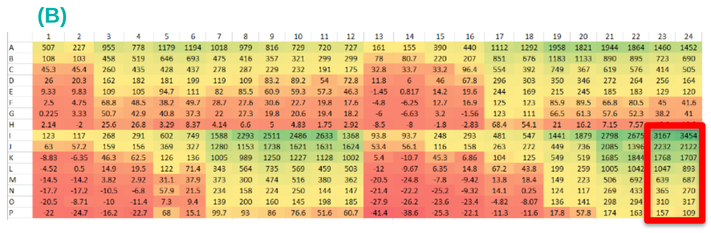


Fig 3. IL18 bead assay optimization with dragonfly discovery showing plate layout (A) and assay readout heatmap (B).

4. bead assay analysis platform

- 96, 384, 1,536 compatible plate reader
- 405nm, 488nm and 640nm lasers
- photomultiplier detection
- no fluidics or cleaning cycles required
- mix and read assay-compatible

5. multiplexed bead assays

- five differently-coded sol-R beads are available
- different capture antibodies can be immobilised to the different bead codes

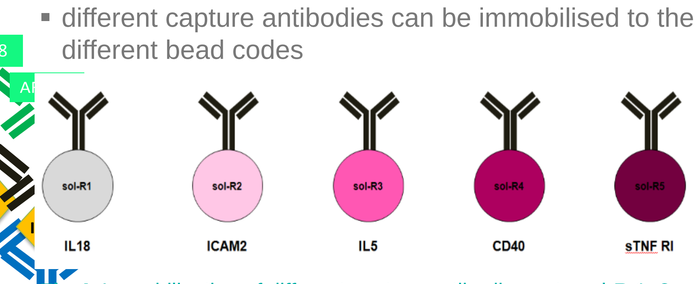


Fig 4. Immobilisation of different capture antibodies onto sol-R 1, 2, 3, 4, and 5 beads.

A multiplexed detection mixture containing capture antibody-coated beads targeted against IL18, ICAM2, IL5, CD40 and sTNF RI, along with biotinylated detection antibodies for all five target proteins and AF488-streptavidin was prepared. This detection mixture specifically detected only the correct target proteins (Fig 5).

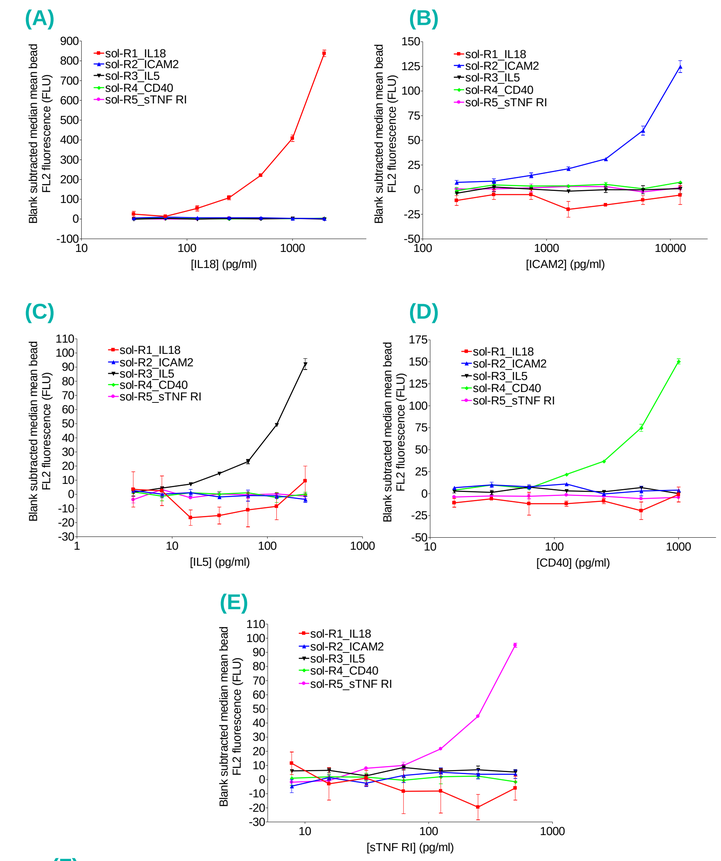


Fig 5. five-plex bead-based cytokine detection showing specific detection of IL18 (A), ICAM2 (B), IL5 (C), CD40 (D) and sTNF RI (E), with assay procedure (F).

Optimisation of singleplex assays on dragonfly discovery allowed rapid identification of detection conditions which could be employed in the multiplex assay format.

conclusions

This novel liquid dispensing technology offers multiple advantages for assay development:

- easily dispense complex plate layouts
- try many more potential reagent concentration combinations than would be possible by hand
- dispense liquids with a range of viscosities and wetting properties using the same instrument
- non-contact dispensing technology and disposable tips prevent cross contamination or carry-over problems
- compatible with many assay formats including bead and cell assays
- compatible with 96, 384 and 1,536 well plates

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