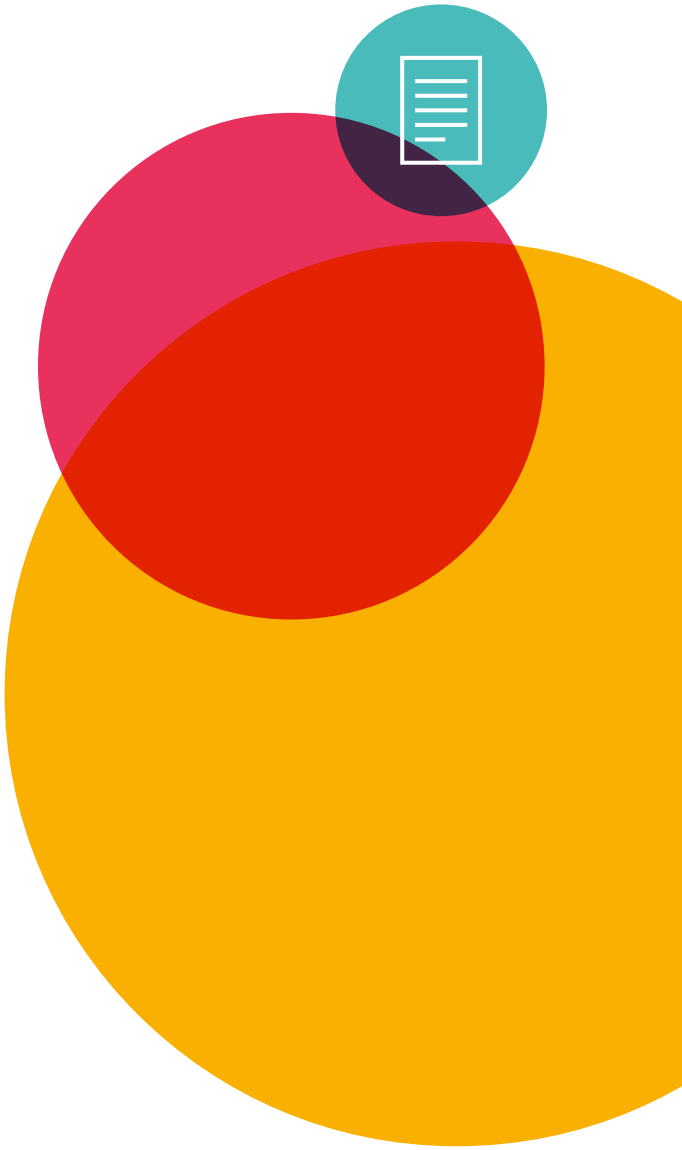
Fast and easy multiplex immunoassay development

Cytokines as an example



Summary

› Fast and easy porting of existing singleplex immunoassays in the development of high performance multiplex Evalution assays
› Fast iteration cycles during assay optimization to obtain market compatible specifications
› Reduced analysis and hands-on times allowed by a unique reaction limited regime within microfluidic channels

MyCartis

BCLA-00344 version 1

Fast and easy porting of existing singleplex ELISA assays into a 50-plex cytokine Evalution assay

MyCartis' Evalution™ analysis platform[1] allows easy porting of existing singleplex sandwich ELISA assays and facilitates subsequent merging into multiplex assays. To demonstrate this, we have chosen off-the-shelf antibody pairs specific for 50 cytokines. All have a proven performance in sandwich ELISA. Using standard assay conditions without reagent or instrument specific optimizations, a functional 50-plex Evalution assay was obtained, with sensitivities in the low pg/mol range and low off-target binding for most [Figure 1]. Further optimization is essential but the scope of this process will be driven by the final intended use of the assay. Factors that should be taken into account include:

› Assay specifications and robustness needed for your sample set (limits of detection, dynamic range…)
› Time to result
› Sample availability and cost
› Cross reactivity issues
› Sample matrix interference
› Reagent cost
› …

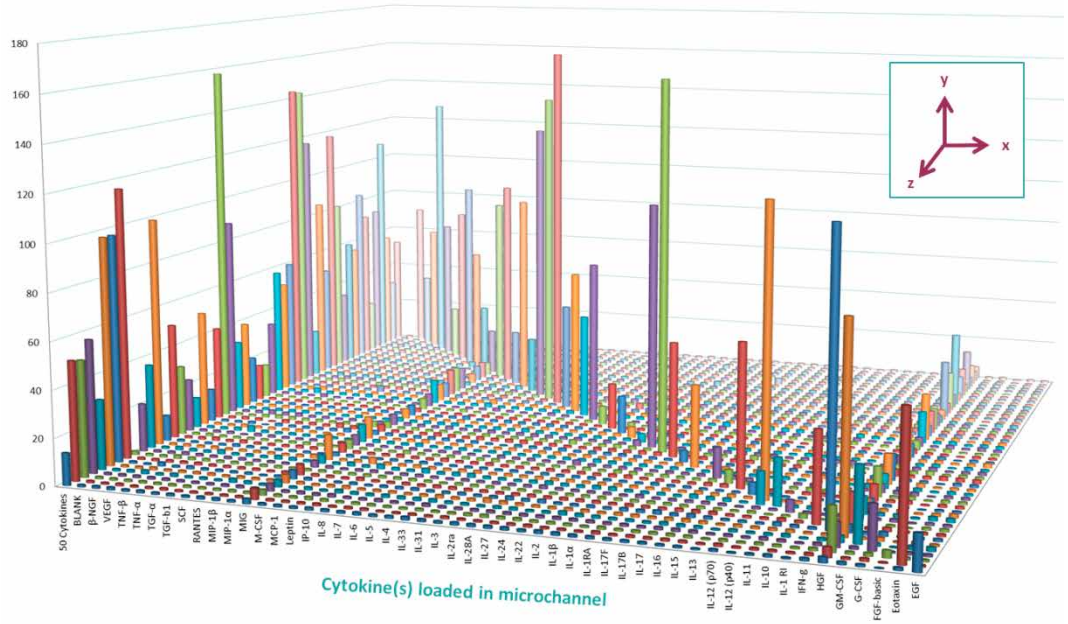


Figure 1: Easy porting of existing ELISA assays into a multiplex format

Readily available antibody pairs with proven performance in ELISA were selected and used to build a 50-plex Evalution assay. Capture antibody coupled microparticles (one microparticle code per capture antibody)(x-axis) were loaded into the microchannels of an Evalution cartridge as a multiplex (z-axis). A sample composed of 33% human serum spiked with a single or all targets (1nM each) was flushed, followed by a mixture of biotinylated detector antibodies and a fluorescent streptavidin tracer. End-point fluorescent measurements are shown (y-axis).

[1] MyCartis' Evalution™ analysis platform is intended for research use only. Not for use in diagnostic procedures.

Optimization steps for improved assay specifications

Optimization procedures that typically lead to improved assay performance are shared with other immunoassay technologies:

› Antibody pair selection [Figure 2a]
› Capture/detection antibody pair orientation
› Capture/detection antibody concentration
› Flush times and flow rates for an optimal analysis time
› Reporter dye concentration
› Blocker selection
› Dilution/wash conditions (detergents, stabilizers...) [Figure 2b]

Importantly, reaction kinetics in an Evalution reaction chamber (i.e. the microfluidic channel filled with static microparticles) is very different from other immunoassay formats. Under optimized conditions, a reaction limited regime characterized by a maximum binding rate is achieved in the microfluidic channels (see also TN005). This results in much reduced assay times and improved binding conditions. Although the optimization steps themselves may be similar among all technologies, the resulting optimal conditions may differ greatly because of this reason.

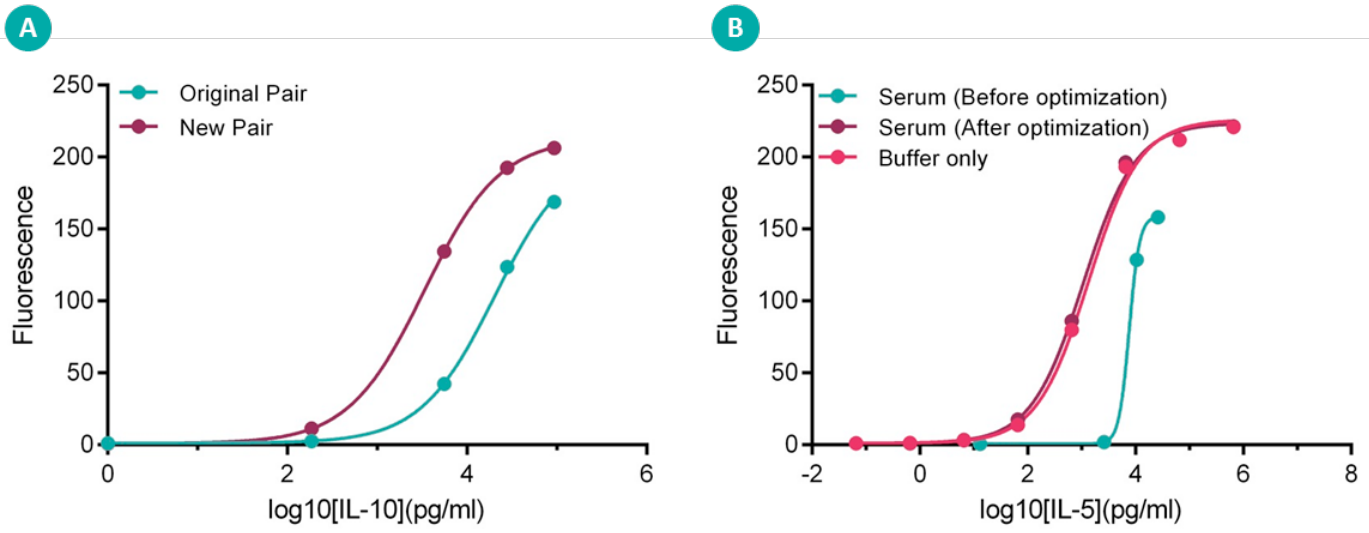


Figure 2: Optimizations improve assay specifications and robustness

Optimization of the standard setup and conditions can drastically improve assay performance. (A) For IL-10, a different antibody pair was selected, immediately resulting in a near 2-log shift in sensitivity. (B) Sample diluent optimization completely solves the issue of sample matrix interference for the IL-5 assay on serum samples.

For a subset of cytokines, a limited (2-month, 1FTE) process to optimize assay specifications resulted in an Evalution multiplex assay with market standard performance: single digit to sub-pg/ml sensitivities, a 5 log dynamic range [Figure 3] and high signal/noise levels at relevant concentrations [Figure 4a]. Excellent specificities could be demonstrated as well as a low impact of the multiplexing environment on the measurements [Figure 4b].

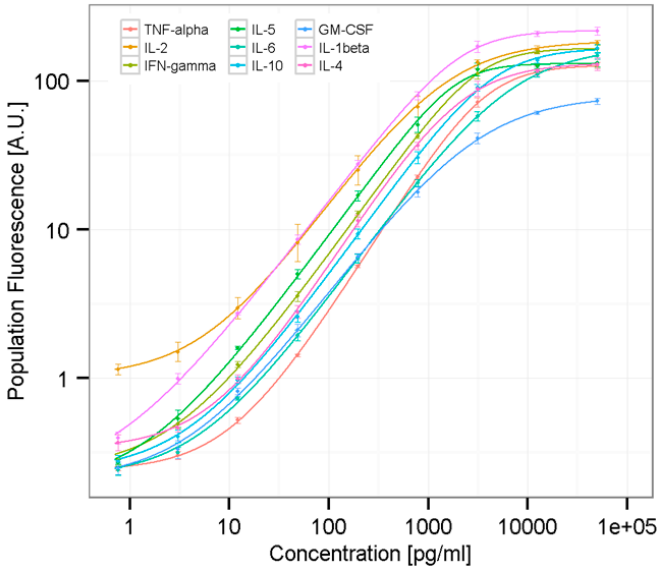


Figure 3: Performance of an optimized multiplex cytokine panel

Standard curves for 9 cytokine assays show single digit pg/ml sensitivities and a dynamic range covering over 5 orders of magnitude. Points and error bars show the result of 4 independent repeats (4 different cartridges run on 4 different days using freshly prepared reagents each time), demonstrating highly reproducible assays for all cytokines included.

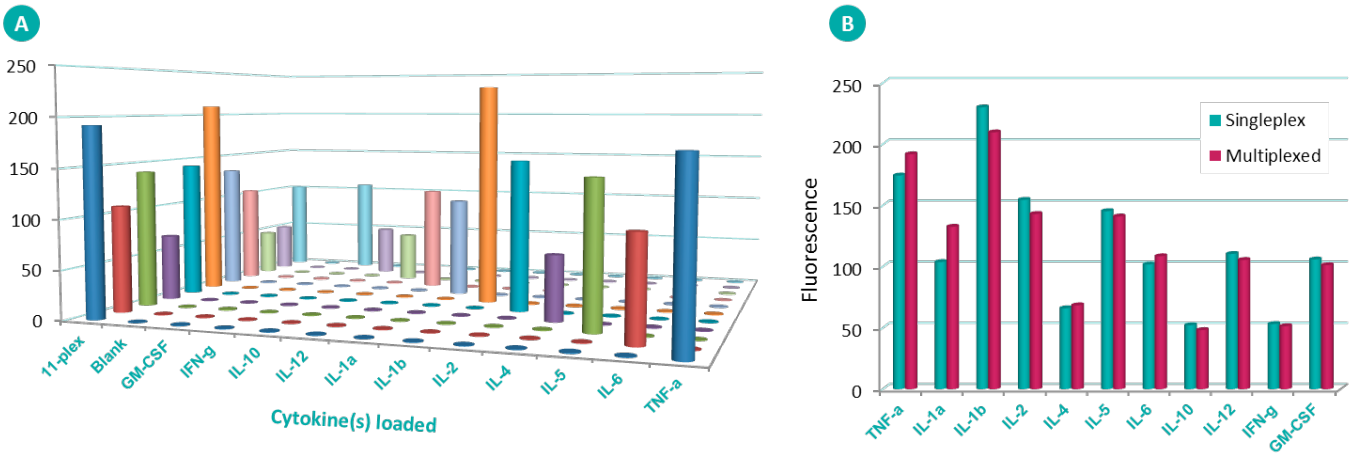


Figure 4: Multiplex assay with singleplex performance

Capture antibody coupled microparticles (one code per capture antibody) (x-axis) were loaded into the microchannels of an Evalution Cartridge as a multiplex (z-axis). A sample composed of 33% human serum spiked with a single or all targets (1nM each) was loaded and the assay run under optimized conditions. (A) End-point measurements show high specificity and low cross reactivity for all assays. (B) No significant effect of the multiplexing process on the single analyte performance could be observed.

Workflow optimizations for shorter total analysis times and less hands-on steps

Most immunoassay formats, single- and multiplex, suffer from slow binding kinetics which results in long incubation and analysis times; typically several hours to overnight sample incubation times are needed to reach sufficient sensitivity. In an Evalution microfluidic channel, the flow guarantees continuous supply of analytes and thus a maximum binding rate is achieved, a condition also referred to as a reaction limited regime. This results in short incubation times and has a direct effect on the total assay times. Next, it has been shown that co-flowing of sample and reagents (like detector antibodies) works perfectly well in the Evalution system. This again drastically reduces total assay times as well as hands-on steps [Figure 5].

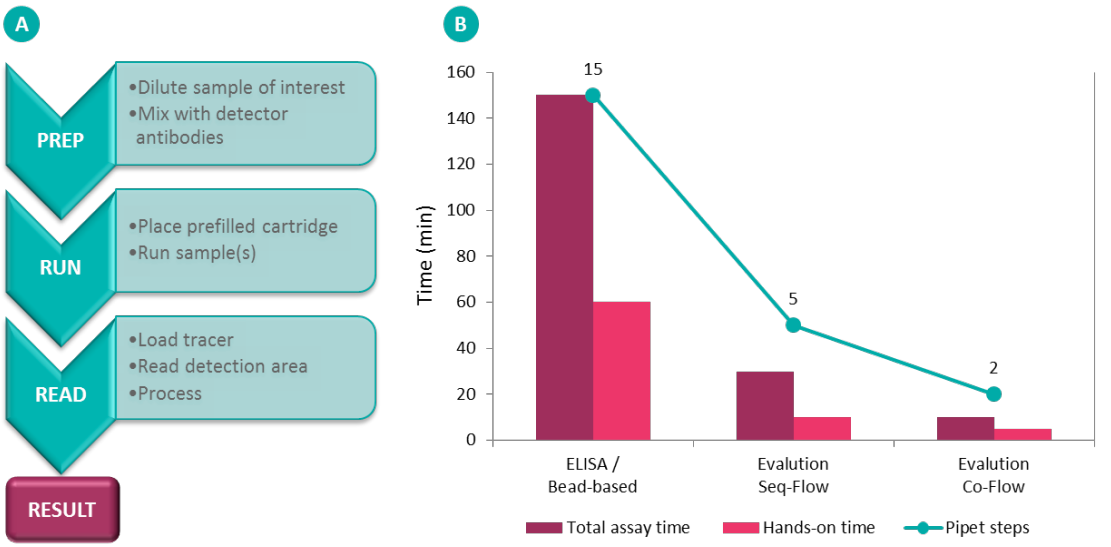


Figure 5: A co-flow Evalution workflow leads to low total assay and hand-on time and assays with minimal manual steps

(A) A typical co-flow Evalution workflow leads to a fast time to result. (B) For a HIV-p24 serological test, a direct comparison with other assay formats was made.

MyCartis at a glance

The big revolution in healthcare today is that we are finally realizing that we are all equal, but not identical. MyCartis is convinced that the future of healthcare lies in personalization. Our goal is to deliver innovative solutions for fast and cost effective identification of a patient's signature. By building bridges between research and medical practices we are increasing the level of health and the quality of life for everyone. We dedicate ourselves to improve healthcare for future generations.

Evalution™ at a glance

› Powerful tool for assay development and biomarker analysis
› Broad range of applications
› Single platform, different analytes
› Simple workflows with minimal handling
› High sensitivity and broad dynamic range for robust measurements
› Competitive cost per data point

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