GYROS PROTEIN Technologies

Extended sensitivity of microfluidic immunoassays for pharmacokinetic (PK) and biomarker analysis

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Background

The use of ligand-binding assays in biotherapeutic development is a mainstay of analytical groups in biopharma companies. Since its introduction, the compact disk (CD) based, nanoliter-scale Gyrolab® microfluidic immunoassay platform has been widely accepted as an essential analytical technology due to its time-saving, automated, and robust performance. The success of the assay format centers around the microfluidic CD labware where the immunoassay takes place. Recently, the Gyrolab Bioaffy™ 4000 CD has been introduced to increase assay sensitivity 2- to 6-fold beyond the current 6-log dynamic range. In this poster, we present data pharmacokinetic (PK) and biomarker analysis down to low pg/mL levels with the Gyrolab Bioaffy 4000 CD demonstrating the sensitivity expansion of Gyrolab immunoassays facilitated with the new CD labware.

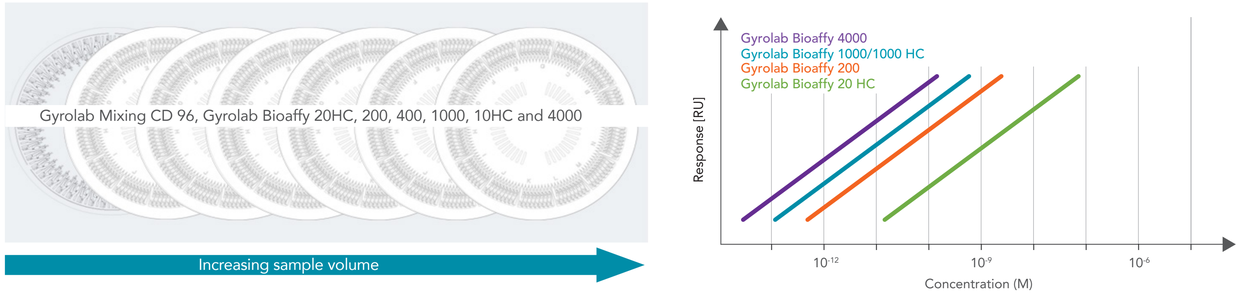
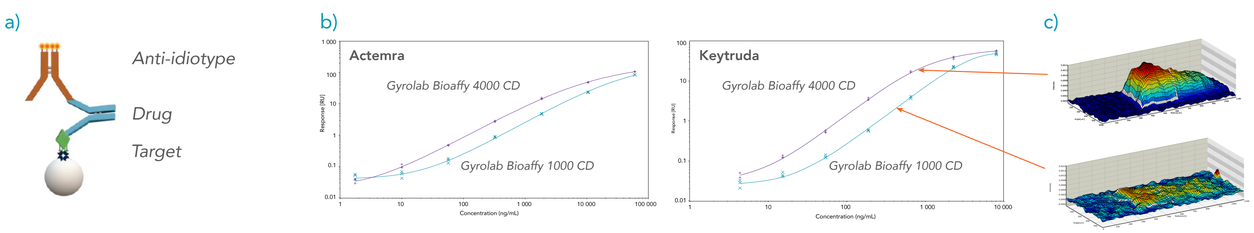


Figure 1. ***Gyrolab Bioaffy CD sample volume capacity correlation to assay sensitivity.*** *By altering the size of the volume definition chamber, or the affinity of the column using high-capacity beads, the assay range provided by the Gyrolab Bioaffy CD family is over 6 logs. This range has been extended with the introduction of Gyrolab Bioaffy 4000 CD.*

Sensitivity of Actemra® and Keytruda® PK assay is extended using Gyrolab Bioaffy 4000 CD



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CD Type	ACTEMRA		KEYTRUDA	
	LLOQ (ng/mL)	ULOQ (ng/mL)	LLOQ (ng/mL)	ULOQ (ng/mL)
1000	60	20,000	18	6,000
4000	20	20,000	6	6,000

Figure 2. ***Actemra® (tocilizumab) and Keytruda® (pembrolizumab) Gyrolab PK assay standard curves.***

a) *Bridging three-step sandwich assay with biotinylated human PD-1 as capture reagent and recombinant human anti-idiotype (pembrolizumab or tocilizumab) labeled with Alexa Fluor® 647 as detection reagent,* ***b)*** *PK assay dynamic range for Actemra (left) and Keytruda (right) using Gyrolab Bioaffy 4000 CD and 1000 CD,* ***c)*** *Viewer profiles of representative data points showing column fluorescence representing analyte binding,* ***d)*** *LLoQ and ULoQ assay results for Actemra and Keytruda. The three-step bridging Gyrolab PK assay was run using Rexxip H with 5% human serum and humanized IgG1 monoclonal antibody tocilizumab or humanized IgG4 monoclonal antibody pembrolizumab as the standard.*

Increased assay sensitivity for cytokine biomarker analysis using Gyrolab Bioaffy 4000 CD

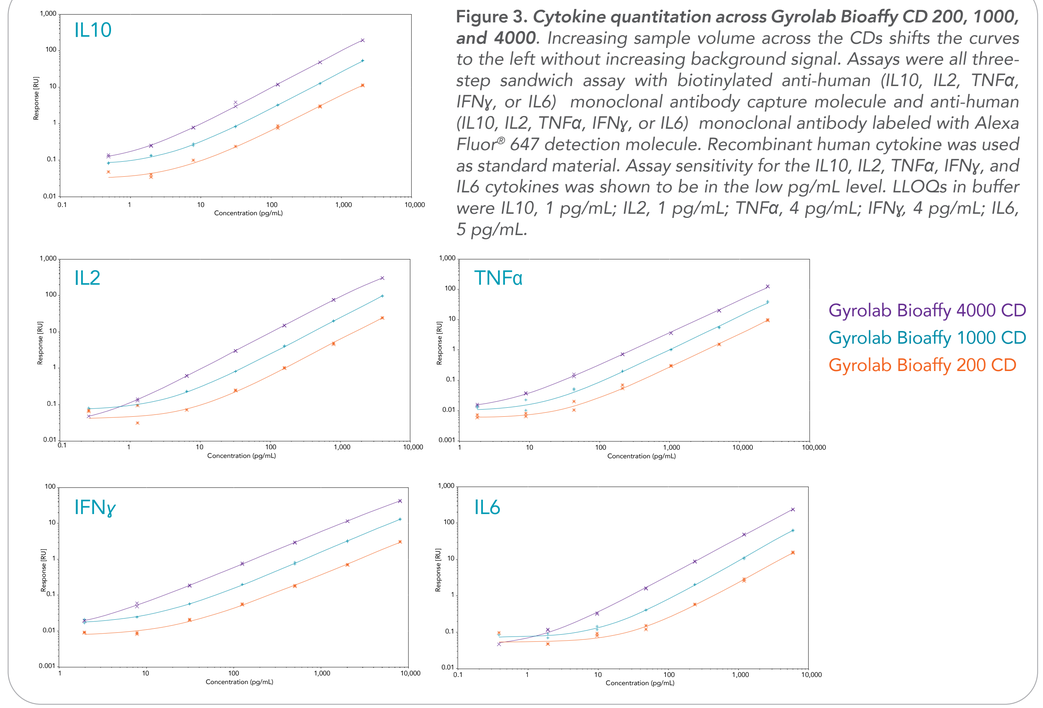

Figure 3. ***Cytokine quantitation across Gyrolab Bioaffy CD 200, 1000, and 4000.*** *Increasing sample volume across the CDs shifts the curves to the left without increasing background signal. Assays were all three-step sandwich assay with biotinylated anti-human (IL10, IL2, TNFα, IFNγ, or IL6) monoclonal antibody capture molecule and anti-human (IL10, IL2, TNFα, IFNγ, or IL6) monoclonal antibody labeled with Alexa Fluor® 647 detection molecule. Recombinant human cytokine was used as standard material. Assay sensitivity for the IL10, IL2, TNFα, IFNγ, and IL6 cytokines was shown to be in the low pg/mL level. LLOQs in buffer were IL10, 1 pg/mL; IL2, 1 pg/mL; TNFα, 4 pg/mL; IFNγ, 4 pg/mL; IL6, 5 pg/mL.*

Summary

Gyrolab Bioaffy 4000 CD has been recently introduced to extend the sensitivity of the Gyrolab Bioaffy CD family by increasing the sample volume in the CD to 4000 nL. PK and biomarker assay results support this extended sensitivity:

- Use of the Bioaffy 4000 CD increased LLoQ of Actemra and Keytruda PK assays from 60 ng/mL to 20 ng/mL and 18 to 6 ng/mL, respectively, demonstrating a 3-fold increase.
- Viewer profiles support increased assay sensitivity with greater fluorescent peaks of analyte bound to the Gyrolab Bioaffy 4000 versus Gyrolab Bioaffy 1000 CD columns.
- Similarly, curves for analysis of IL10, IL2, TNFα, IFNγ, and IL6 cytokines were shifted to the left, indicating increased assay sensitivity, with LLoQ values in the low ng/mL range.
- Background signal was not increased with the larger sample volumes of the Gyrolab Bioaffy 4000 CD in any of the PK and biomarker assays shown.

This extended sensitivity will be useful for PK and biomarker studies requiring higher sensitivity using Gyrolab platform, while maintaining the high reproducibility and broad dynamic range that Gyrolab microfluidic assays provide.

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