

Rapid and sensitive multi-biomarker analysis powered by Gyroplex™ panels on Gyrolab® Bioaffy™ 4000 CD

Application Note

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The increasing value of biomarker panels

Biomarker research has accelerated in recent years, driving the development of novel biomarker candidates and panels for the study of a wide range of diseases. For example, inflammatory cytokines can be used to diagnose or monitor inflammatory disease and may provide clinically applicable parameters for therapies. A case in point is the cytokine storm that can contribute to the development of acute respiratory distress syndrome (ARDS) in patients suffering from COVID-19. Another example is cytokine release syndrome (CRS), a potentially serious complication of chimeric antigen receptor T cell (CART) therapy used in treating cancer. The need to analyze biomarkers such as those included in cytokine panels in biotherapeutics and cell and gene therapy R&D has powered the development of highly sensitive immunoassays that use minimal amounts of precious sample.

How to achieve multiple biomarker analysis: multiplex or singleplex?

Starting with a series of optimized immunoassays, it can be tempting to scale up biomarker analysis by multiplexing. This approach can cut the cost per data point and reduce sample handling, which in turn lowers the risk for error and simplifies lab work.

There are, however, a number of disadvantages inherent in the complexity of multiplexing immunoassays that make efficient singleplex an attractive alternative. One of the biggest obstacles with multiplexing is the widespread level of cross-reactivity between antibody reagents that, together with many other factors, can lead to long assay development times. Assay developers therefore often turn to external providers for less-than-optimal fixed assay panels rather than developing assays in house to meet specific needs. There are also challenges in measuring different analytes over widely different ranges in the same reaction vessel, which demands time-consuming sample dilutions to get all analytes in range. Then there is the question of the “right number” of analytes to measure in a multiplex assay. The limit is around 4–6, above which failure of a single assay can involve a lot of wasted time, sample and reagents. These drawbacks suggest that it can be more productive to run time- and volume-efficient singleplex assays.

Gyroplex™ panels multiply efficient singleplex immunoassays

Running Gyrolab® assays in series on separate CDs is an efficient way to generate more reliable biomarker data from samples. This approach, which we call the Gyroplex panel, has a number of advantages in biomarker analysis:

- No cross-talk or reagent interference: each data point is generated from a dedicated microstructure.
- Well-optimized and individually tailored assays (format, dilution buffers) in a single run ensure panel flexibility.
- In-house, novel or proprietary assays can be readily included.
- The Gyroplex panel is created as part of the immunoassay workflow.
- Each assay can be individually validated.
- There is no need to rerun all assays if one fails.
- The need for only small sample volumes supports preclinical studies.

Increased biomarker assay sensitivity with Gyrolab Bioaffy 4000

The advantages of Gyroplex biomarker panels can be further enhanced by using Gyrolab Bioaffy 4000 CD, which delivers a higher sensitivity than, for example, Gyrolab Bioaffy 1000.

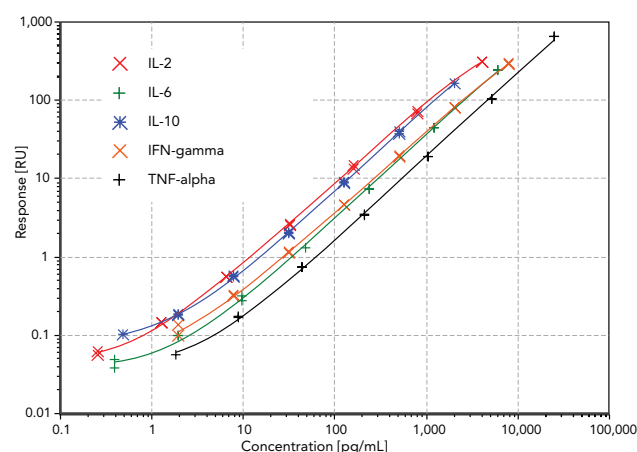


Figure 1. A Gyroplex panel of five cytokine biomarkers (IL-2, IL-6, IL-10, IFN-gamma and TNF-alpha) was run overnight in 5 Gyrolab Bioaffy 4000 CDs on Gyrolab xPand system. All assays had a broad analytical range.

Gyrolab panels enable efficient biomarker analysis

Gyrolab assays are widely known to be robust, have a broad dynamic range, and use very small amounts of precious sample. Added to that, the ability to run individual immunoassays in separate, dedicated microstructures means that data points can be generated without the risk of cross-talk using an assay that is run under optimal conditions. Assays can then be combined in a completely flexible way

to design biomarker panels that meet specific and dynamic needs. Expanding this capability to multiple CDs run overnight on Gyrolab xPand system means that 5 biomarkers can be measured in duplicate in 37 samples. The maximum capacity is 2 x 5 CD runs per 24 hours, with, for example, one Gyrolab Bioaffy 4000 CD taking approximately 80 minutes to run. This enables 370 biomarker levels to be determined in 24 hours.

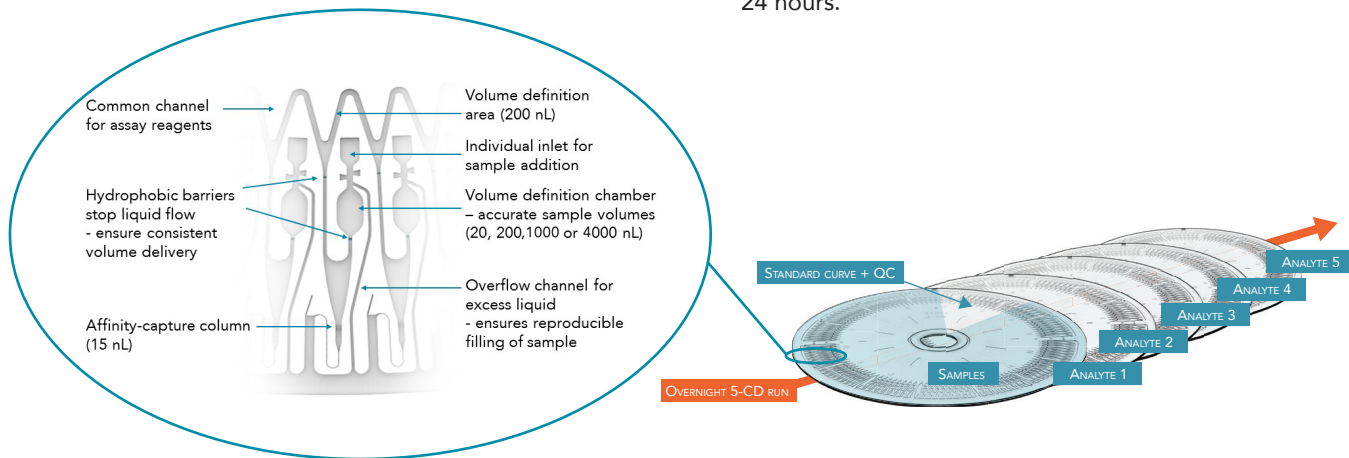


Figure 2. Gyroplexing on a series of five CDs run overnight. The individual microstructures on each CD ensure that each data point is generated with no risk of cross-talk. A total of 37 samples can be run per CD, together with calibrators, a blank, and three QC's, all in duplicate.

Gyrolab Bioaffy 4000 increases immunoassay sensitivity

Gyrolab Bioaffy 4000 CD is based on the industry-proven Gyrolab Bioaffy 1000 CD and has been developed to increase assay sensitivity. By using a deeper volume definition structure (Figure 2) a greater volume of sample can be passed over the streptavidin bed to enable the capture of analyte from low concentration samples. The result is an approach based on proven technology that increases immunoassay sensitivity by 2–6 fold compared with Gyrolab Bioaffy 1000 CD. Adding Gyrolab Bioaffy 4000 CD to the Bioaffy range of CD options has extended the analytical range of Gyrolab system assays even further (Figure 3). Independent evaluation of assay performance using a range of different CDs indicates that Gyrolab Bioaffy 4000 CD can meet the most challenging sensitivity goals for biopharmaceutical R&D. These results are obtained from a single automated 5 CD run.

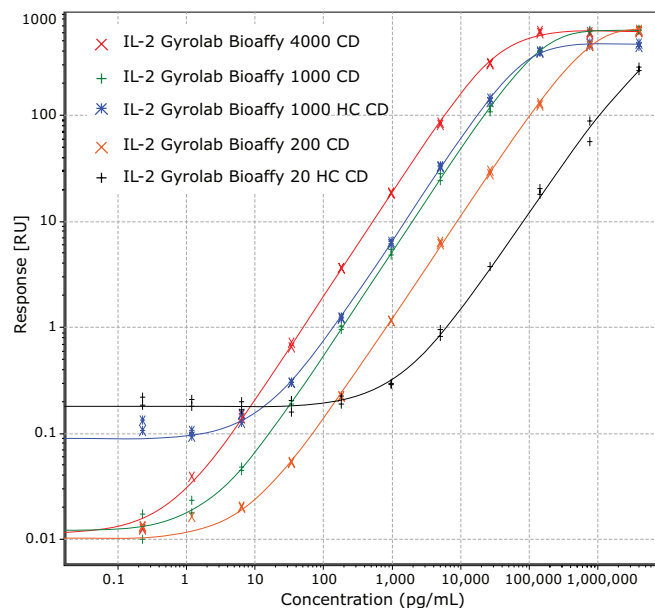


Figure 3. Assay of IL-2 run on the complete range of Gyrolab Bioaffy CDs. Gyrolab Bioaffy 4000 extends the sensitivity of the Gyrolab Bioaffy range from pg/mL down to low pg/mL levels.

The higher binding capacity of CDs using the high capacity (HC) streptavidin particle increases the upper limit of detection but may also increase background binding.

Application: Cytokine panel to follow TH1 response

To illustrate the power of running Gyroplex panels using Gyrolab Bioaffy 4000 CD, five cytokine biomarkers were analyzed by running each assay on a dedicated CD in an overnight run on Gyrolab xPand system with cooling to preserve reagents, samples and standards.

The panel included five cytokines involved in the TH1 response: IL-2, IL-6, IL-10, IFN-gamma and TNF-alpha. An example of the assay design for IL-6 and the resulting standard curve is shown in Figure 4 and Figure 5. Nine serum pool samples were spiked with different concentrations of each analyte and analyzed in four dilutions.

Each assay was run according to the established protocols available at <https://www.gyrosproteintechnologies.com/gyrolab-assays> on Gyros Protein Technologies website and included a standard curve.

Example of cytokine panel assay: IL-6

Assay design

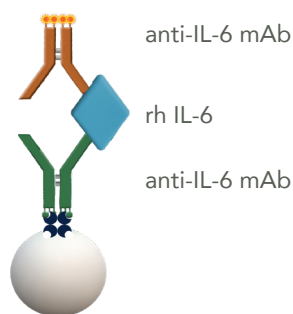


Figure 4. The IL-6 assay was set up as a three-step sandwich immunoassay with biotinylated anti-human IL-6 monoclonal antibody as a capture molecule and an anti-human IL-6 monoclonal antibody labeled with Alexa Fluor® 647 as a detection molecule. Recombinant human IL-6 (rh IL-6) was used as standard material.

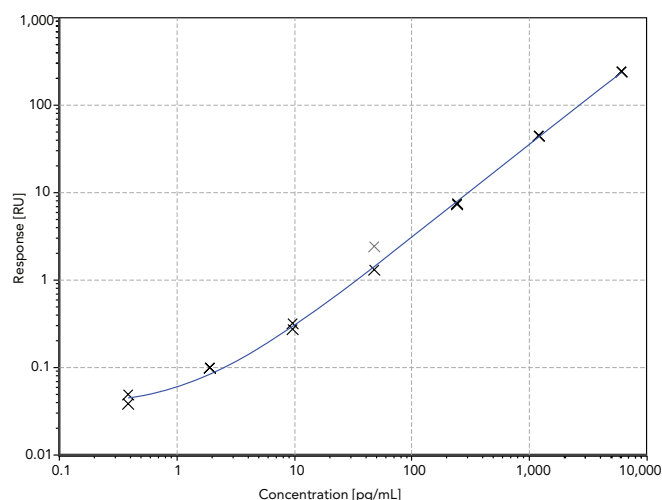


Figure 5. Standard curve for IL-6 assay in Rexxip H. The assay range obtained was 5–5500 pg/mL, i.e. over 3 logs. This broad dynamic range can be of great value when analyzing samples where concentrations can increase from picomolar- to nanomolar levels during trauma or infection.

The inter-run precision of IL-6 quantitation (CV, Coefficient of Variation), established with QC samples over the assay range run in triplicate in three runs, was <20% (Table 1).

Table 1 Accuracy and precision data of QC samples in Rexxip H, n = number of runs

QC	Expected Conc (pg/mL)	Average Measured Conc (pg/mL)	Inter Run CV (%; n=3)	Average Intra Run CV (%; n=3)	Average Total Error (%; n=3)
1	5	5.1	2.6	13	16
2	10	10	12	7.4	16
3	25	28	6.5	3.0	14
4	100	94	5.5	4.1	9.7
5	2 000	1 941	6.7	3.7	9.6
6	4 000	3 770	3.6	2.5	8.2
7	5 500	5 478	2.3	2.5	4.4

Assay performance of the 5-cytokine Gyroplex panel

The overnight run was used to determine the assay performance of the individual assays, including the dynamic range, spike recovery and dilutional linearity.

Dynamic range

The broad dynamic ranges of the individual assays in the 5-cytokine Gyroplex panel based on Gyrolab Bioaffy 4000 enable the efficient determination of biomarker levels that can vary from normal endogenous levels to raised levels resulting from disease (Figure 1). For example, normal levels of IL-6 can be in the order of 0–20 pg/mL but can rise to over 200 pg/mL in the case of the cytokine storm associated with severe COVID-19 (1), and as high as 10000 pg/mL in the case of severe illness, such as preterm neonates with sepsis (2).

Spike recovery

To determine the accuracy of the individual assays, nine samples were spiked with pg/mL- and ng/mL levels of each biomarker. The results for a single sample for all five cytokines demonstrate good linearity over the dilutional ranges (Figure 6) with recoveries that fall within acceptance criteria (+/- 20%) (Table 2). Good dilutional linearity reduces the need for sample dilution and therefore ensures a sensitive assay.

Table 2. Excellent recovery was obtained from high and low cytokine levels (given in pg/mL). Unspiked samples measured below LLOQ (Lower Limit of Quantification) confirming the selectivity of the assays.

Sample	IL-2			IL-6			IL-10			IFN-gamma			TNF-alpha		
	Expected conc. (pg/mL)	Average conc. (pg/mL)	Recovery (%)	Expected conc. (pg/mL)	Average conc. (pg/mL)	Recovery (%)	Expected conc. (pg/mL)	Average conc. (pg/mL)	Recovery (%)	Expected conc. (pg/mL)	Average conc. (pg/mL)	Recovery (%)	Exp conc. (pg/mL)	Average conc. (pg/mL)	Recovery (%)
1	49.7	52.5	106	135	146	108	2 045	2 115	103	10 319	10 669	103	5 202	5 818	112
2	4 686	5 518	118	10 400	11 236	108	50.5	55.3	109	139	141	101	135	149	110
3	4 686	5 472	117	135	149	110	0	0*	N/A	10 319	10 727	104	135	142	105
4	49.7	53.3	107	10 400	10 834	104	50.5	56.0	111	139	154	111	5 202	5 551	107
5	49.7	53.0	107	0	0*	N/A	50.5	54.5	108	10 319	10 881	105	5 202	5 505	106
6	4 686	5 347	114	10 400	10 043	96.6	2 045	1 975	96.6	139	144	103	135	134	98.9
7	0	0*	N/A	135	151	112	2 045	1 915	93.7	0	0*	N/A	0	0*	N/A
8	4 686	5 431	116	10 400	11 006	106	2 045	1 947	95.2	10 319	10 185	98.7	5 202	5 268	101
9	49.7	51.2	103	135	141	105	50.5	49.1	97.2	139	142	102	135	125	92.2

*Concentration measured below LLOQ.

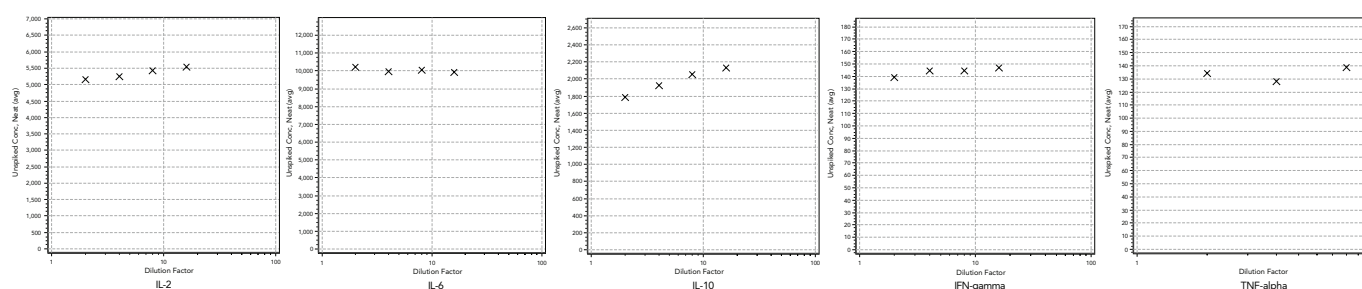


Figure 6. Dilutional linearity for analysis of all five cytokines in one sample run as Gyroplex panel. Concentrations given in pg/mL. One dilution for TNF-alpha was out of range. The results for a single sample for all five cytokines demonstrate good linearity over the dilutional ranges.

Conclusions

Gyroplex panels enable multiple biomarkers to be analyzed using very low volumes of samples. A maximum of 12 µL (for a duplicate sample) sample is required per assay to analyze a sample. Less original sample is used if diluted. Another advantage of Gyroplex panels over multiplexing is that samples can be diluted in the buffer that is optimal for the particular biomarker assay. The possibility of running individual optimized assays with no cross-talk between assays or samples enables the confident analysis of different analytes with widely different concentrations in the same run, optimized to the requirements of the biomarker assay. This approach provides the flexibility during the development of biomarker panels and avoids the complexity and inflexibility of multiplexing, where a single set of assay conditions is required for all analytes.

Overnight runs can boost throughput to approximately 370 biomarker levels in 24 hours. Gyrolab platform is open to development of in-house assays, which means your analytical possibilities are only limited by your imagination and access to suitable reagents. Added to that, combining Gyroplex panels with Gyrolab Bioaffy 4000 CD means that goals for assay sensitivity can be met when running biomarker panels such as cytokines.

References

1. Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. Huan Han *et al.* (2020), *Emerging Microbes & Infections*, 9:1, 1123-1130.
2. Plasma levels of interleukin-6 and interleukin-10 in preterm neonates evaluated for sepsis. Romagnoli, C. *et al.* *Eur J Pediatr* (2001) 160: 345–350.

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