Increased sample volume improves ability to quantify cytokines in Bioaffy 1000

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Conclusions

- Gyrolab® is an automated quantitative screening system
- Bioaffy® 1000 is well suited for biomarker quantification
- Assay conditions in Gyrolab can be adapted to suit analytical needs
- Gyrolab allows for making best use of small sample volumes

Introduction

Gyrolab is a scalable and transferable platform for analyte quantification. The miniaturized and automated format offers inherent advantages over established technologies such as ELISA, providing benefits in terms of workflow effectiveness and improved assay performance.

Tailoring the Gyrolab platform for use in biomarker research, detection limits have been further improved through the development of Bioaffy 1000, processing 1000 nL of sample in each immunoassay. The performance of Bioaffy 1000 has been evaluated for different analytes in human serum and plasma samples.

Gyrolab Technology

The Gyrolab platform utilizes disposable CDs containing individual microstructures in which samples are analyzed in parallel. Each microstructure contains a 15 nL column of streptavidin coated particles on which reactions take place. The principles for miniaturizing and integrating assays on Bioaffy CD microlaboratories are illustrated in Figure 1.

CD microlaboratories are run in Gyrolab (see Figure 3). Samples and reagents are transferred sequentially to specific inlets on the CD by a robotic arm. Liquid enters the microstructures by capillary action and the required volumes are defined within each microstructure. Samples and reagents are moved through the microstructure by spinning the CD at pre-programmed intervals and speeds to create the desired flow rates. Detection begins as soon as the reactions are completed using a laser-induced fluorescence detector integrated in the workstation. Thus, an image of the localized fluorescence intensity of each individual column is created. The total integrated fluorescence of each column corresponds to the total protein bound to the column (see Figure 2 a, b). Each Bioaffy 1000 CD takes approximately one hour to process and generates 96 data points.

Assays are created by sequential addition of reactants and processed in flow-trough mode. All interactions occur under flow conditions reducing non-specific interactions favoring high signal:noise ratios and wide assay working ranges. The open nature of the platform combined with rapid processing makes it applicable for various analytical situations.

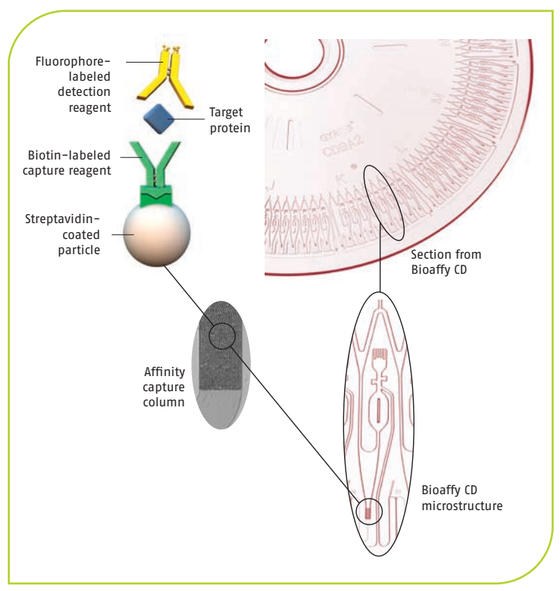

Fig. 1. Sandwich-based immunoassays miniaturized and integrated into a CD microlaboratory.

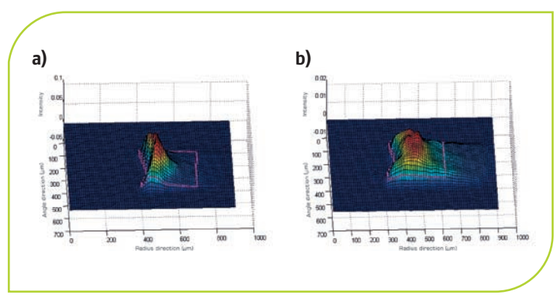

Fig. 2. Gyrolab Viewer graphically displays the fluorescence data from each column, providing more qualitative data regarding affinity and unspecific interactions. Shown here is the same concentration of Tissue Factor assayed with two different reagent combinations, Ab1/Ab2 (2b) and Ab3/Ab1 (2a). The combination Ab3/Ab1 shown in Figure 2a) is more suitable for a high sensitivity assay required to work at physiological concentrations.

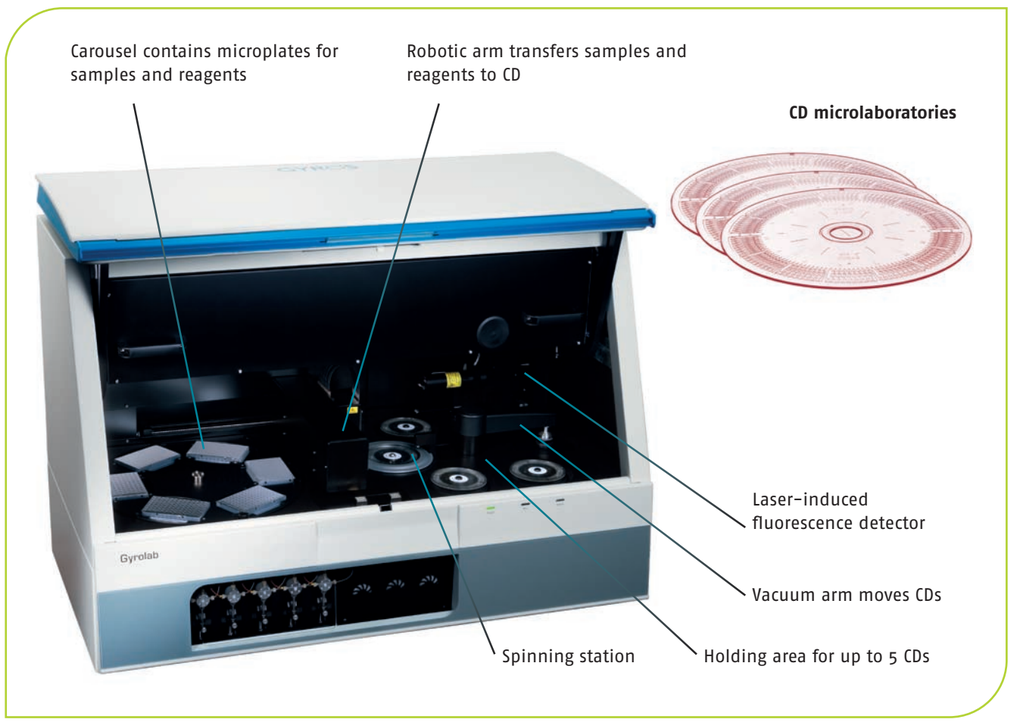

Fig. 3. Gyrolab.

Experimental

Serum and plasma samples were collected from 68 blood donors and 23 patients. Samples were analyzed with respect to MIP-1β and Tissue Factor concentration.

Sample and reagent volumes required to quantify two biomarkers in duplicate using Gyrolab Bioaffy 200 and Gyrolab Bioaffy 1000 respectively are shown in Table 1. For comparison, the corresponding values for a typical ELISA are included.

Table 1. Required volumes for duplicate measurements of two biomarkers

	Bioaffy 200	Bioaffy 1000	Typical ELISA
Sample	4.5 µl	15 µl	200 µl
Reagent	5 µl	5 µl	70 µl

Results

Increased sensitivity with Bioaffy 1000

In a dynamic range comparison, standard curves for MIP-1β were prepared using Bioaffy 200 and Bioaffy 1000 respectively. As shown in Figure 4, the increase in sample volume results in a higher signal with no significant increase in background. Thus, with the introduction of Bioaffy 1000 the dynamic range of the assay is shifted towards lower concentrations, decreasing the limit of detection by a factor of four.

Serum and plasma samples from blood donors and patients were analyzed with respect to Tissue Factor concentration. Samples were processed in Bioaffy 200 and Bioaffy 1000 respectively. As expected, the concentration levels in most of the samples were too low to enable quantification, i.e. a signal:noise ratio of three or more, using Bioaffy 200 (Figure 5). When the same samples were analyzed in Bioaffy 1000 quantifiable results were achieved for 92% of the samples.

Similar results were achieved when quantifying MIP-1β. The resulting concentration range of MIP-1β in the serum and plasma samples was 50–900 ng/ml (Figure 6), with a resulting average CV% of 4.3% for triplicate measurements using Bioaffy 1000.

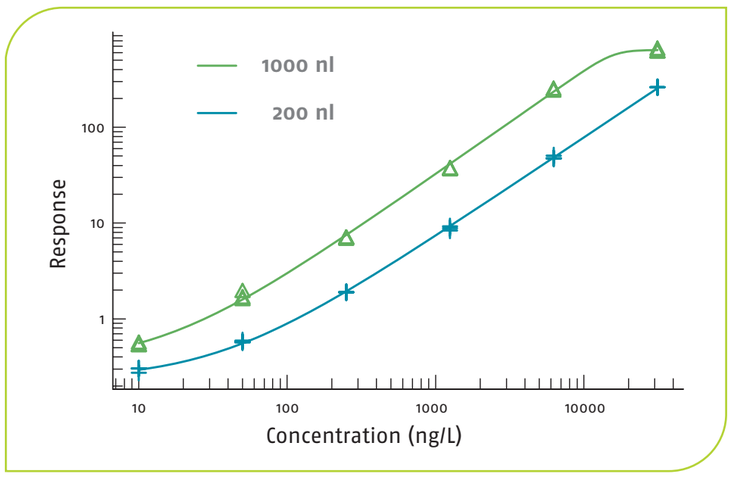

Fig. 4. Effect of increased sample volume. MIP-1β standard curves run on Bioaffy 200 and Bioaffy 1000 respectively.

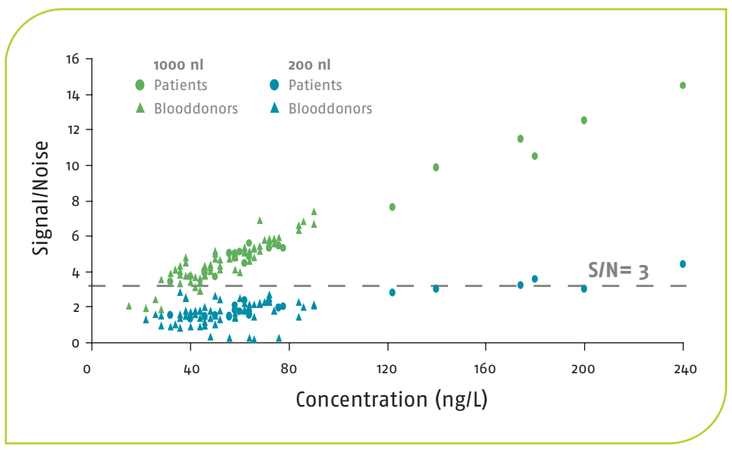

Fig. 5. Tissue Facture measured in serum and plasma from blood donors and patients using Bioaffy 200 and Bioaffy 1000 respectively.

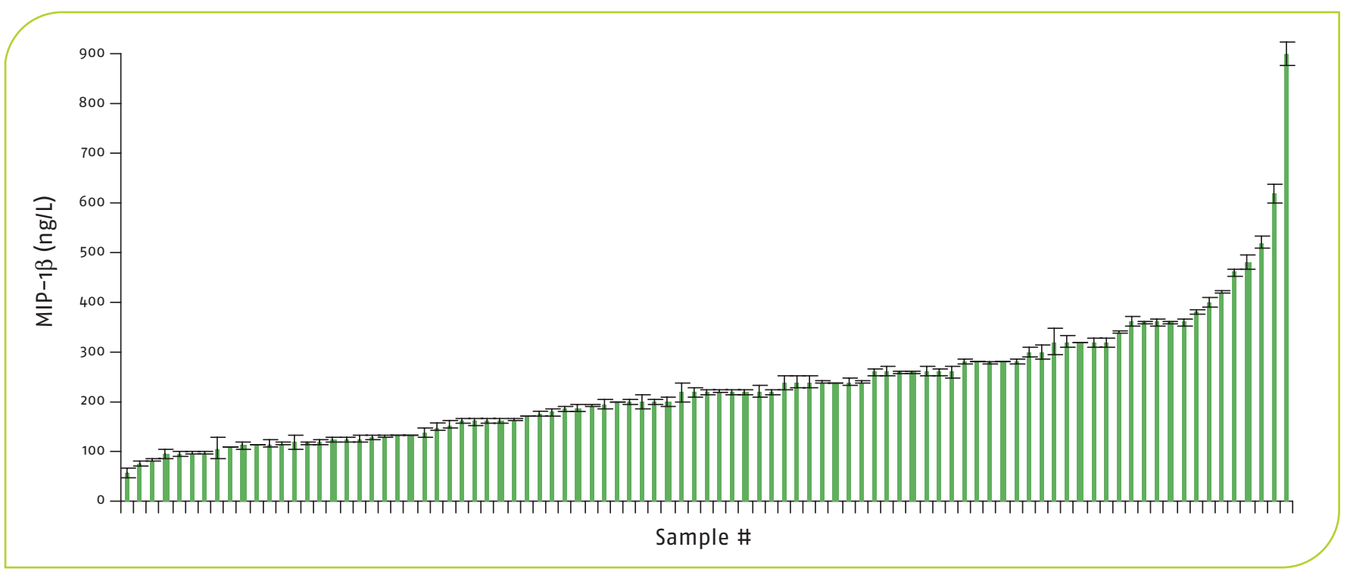

Fig. 6. MIP-1β measured in serum and plasma from blood donors and patients using Bioaffy 1000. Resulting average CV% for triplicate measurements was 4.3%.

Importance of immunoreagents

The assay for human Tissue Factor illustrates the impact of the immunoreagents on assay performance. In this case three antibodies Ab1, Ab2, Ab3 were used in combinations Ab1/Ab2 and Ab3/Ab1. The resulting standard curves are shown in Figure 7, with the corresponding Gyrolab Viewer images shown in Figure 2. The Gyrolab Viewer images confirm that the binding characteristics of the combination in Figure 2a) are more suitable for a high sensitivity assay required to work at physiological concentrations.

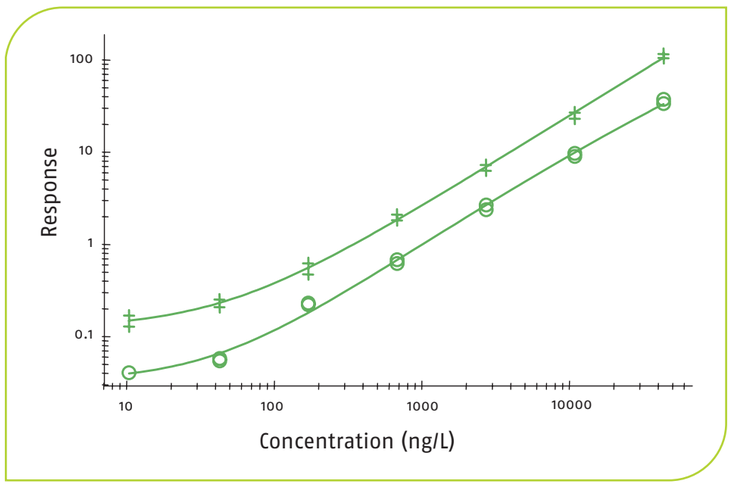

Fig. 7. Illustration of the importance of immunoreagents for assay performance. Tissue Factor standard curves for two different reagent combinations, Ab1/Ab2 (lower) and Ab3/Ab1 (upper).

Acknowledgements

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