Miniaturized protein microarray with internal calibration for diagnosis of neonatal sepsis

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Introduction

☐Sepsis is a life-threatening systemic body infection and still a leading cause of death among newborns. Symptoms are often wrongly interpreted due to their late and unspecific occurrence. Therefore early-stage diagnosis is of tremendous importance enabling timely administration of antibiotics. For this purpose a protein microarray that allows the simultaneous detection of the sepsis associated serum proteins IL-6, IL-8, IL-10, TNF alpha, CRP, PCT, neopterin, S-100 and E-Selectin has been developed. To keep invasive procedures on neonates as low as possible the protein chip was miniaturized. Whereas the classical assay format requires 20 µl patient sample the new low volume assay works with 4 µl patient serum sample. Assay miniaturization provokes losses in assay sensitivity due to diffusion limitations. Therefore streptavidin coated magnetic particles are added which act as micro-mixer and at the same time detect biotinylated antibodies. Optimization of the miniaturized protein microarray focused on the simplification and acceleration of the assay procedure by reducing assay steps and establishing an internal calibration.

Classical versus miniaturized chip format

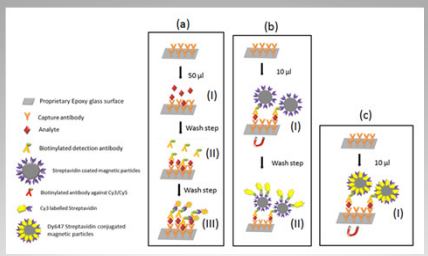

Figure 1. On-chip sandwich immunoassay steps for **(a)** the classical chip **(b)** the miniaturized chip and **(c)** the optimized miniaturized chip

The differences between the classical and the miniaturized chip formats are outlined in figure 1. Specific antibodies for sandwich immunoassays and antigens for binding inhibition assays are immobilized on ARChip Epoxy supported platforms [1]. Figure 1a shows the classical chip where 20 µl serum sample diluted with 180 µl assay buffer are incubated and analytes are detected by the incubation of biotinylated detection antibodies and fluorescence labeled streptavidin [2]. Figure 1b presents the miniaturized chip format. Only 4 µl serum sample diluted with 36 µl assay buffer as well as biotinylated antibodies and streptavidin coated magnetic particles (strept. MPs) are incubated and analytes are detected by the incubation of biotinylated antibody against Cy3/Cy5 and Cy3 labeled streptavidin.
To simplify and accelerate the miniaturized assay format strept. MPs were fluorescence labeled which replaces the second incubation step as shown in figure 1c. In this way an „all-in-one-step" assay that accomplishes within 2.5 hours was created .

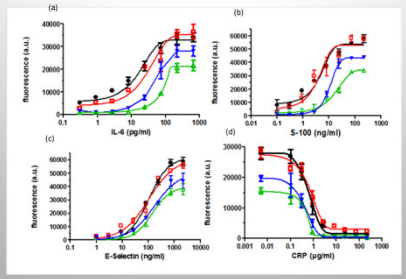

Figure 2. Calibration curves for **(a)** IL-6 **(b)** S-100 **(c)** E-Selectin and **(d)** CRP using the classical chip (), the miniaturized chip (), the classical chip + strept MPs () and the miniaturized chip + strept. MPs ()

Figure 2 shows the assay performance of the classical and the miniaturized chip with and without strept. MPs respectively. We demonstrated that assay miniaturization causes losses of sensitivity which is probably due to diffusion limitations. The addition of strept. MPs that act as distinct stirring and detection components significantly increases assay sensitivity as outlined in figure 2 providing an accurate and reliable low volume tool for diagnosis of neonatal sepsis.

Internal calibration

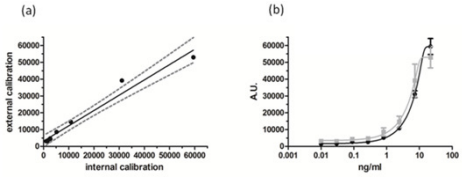

Figure 3. Scatter plot **(a)** and calibration curves **(b)** for the internal () and external calibration () for S-100

For calibration of the protein microarray a standard dilution series has to be prepared for each assay (external calibration). To reduce the effort and further simplify the protein-chip the calibration was directly integrated on the chip. S-100 was conjugated to BSA and spotted in increasing concentrations to form an internal calibration. Figure 3a depicts the correlation and precision between the internal and external calibration. A strong linear correlation is observed and most values cluster within the 95 % confidence band of the fit line. Figure 3b shows the internal and external calibration curves which are almost indistinguishable from each other in terms of curve shape as well as maximal signal intensities.

Conclusion

We developed a protein microarray that fulfills requirements for point-of-care diagnostics in terms of miniaturization, assay time and internal calibration. The optimized low volume chip is especially attractive for diagnosis in neonates since the reduced sample volume minimizes stress and pain caused through excessive blood withdrawal.

Acknowledgements

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REFERENCES

[1] US patent, no. 7790472
[2] Sauer, U.; Domnanich, P.; Preininger, C. Protein chip for the parallel quantification of high and low abundant biomarkers for sepsis. *Anal Biochem* **2011**, 419, 46-52.