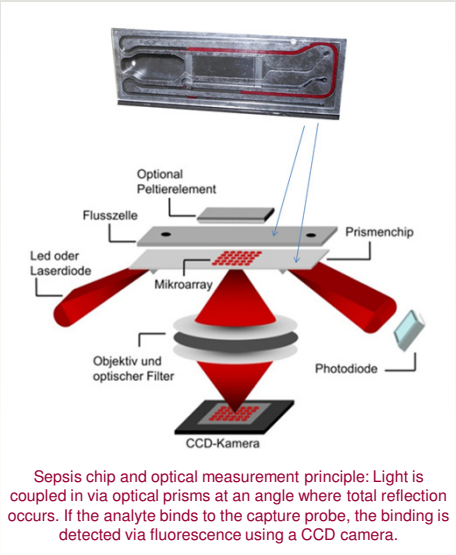
Valipro - Prototype development and validation of a TIRF based protein microarray system for diagnosis of sepsis

[1]Preininger, C.; [1]Buchegger, P.; [2]Kemmler, M; [2]Brandenburg, A.; [3]Zengerer, R.; [3]Bernhard, T.; [3]Riedlhuber, H.; [4]Engelke, R.; [4]Heinrich, M.; [5]Pfäfflin, A.

claudia.preininger@ait.ac.at, www.valipro.eu

[1] AIT Austrian Institute of Technology GmbH, Health & Environment Department, Tulln, Austria
[2] Fraunhofer Institute of Physical Measurement Techniques, Freiburg, Germany
[3] PAYER International Technologies GmbH, St. Bartholomä/Graz, Austria
[4] micro resist technology, Berlin. Germany
[5] A. Pfäfflin, MVZ Labor PD Dr. Volkmann und Kollegen GbR, Karlsruhe



Sepsis chip and optical measurement principle: Light is coupled in via optical prisms at an angle where total reflection occurs. If the analyte binds to the capture probe, the binding is detected via fluorescence using a CCD camera.

INTRODUCTION

Sepsis is a life-threatening systemic body infection manifested by symptoms ranging from rapid heartbeat, breathing difficulties and fever to severe necrosis of internal organs. It may lead to shock, multiple organ failure, and death, especially if not recognized early and treated promptly. Between one third and one half of patients with sepsis die. In the developing world, sepsis accounts for 60-80% of lost lives per year, killing more than 6 million neonates and children yearly. The incidence of sepsis is increasing dramatically due to an ageing population. International and national surveys indicate that 20-40% of sepsis patients that require treatment in the intensive care unit, developed sepsis outside the hospital (http://www.globalsepsisalliance.org/). Sepsis is caused by microbial pathogens, mostly bacteria. With about 300.000 cases of severe sepsis per year the impact on health economy is huge: the annual cost to Europe as a whole has been estimated to be approximately € 7.6 billion. Thus patient early stage diagnosis is of critical importance to allow timely administration of antibiotics and intensive-care treatment and reduce the incidence of severe sepsis.

THE INSTRUMENT

We have developed and validated a compact, fully automated total internal reflectance fluorescence (TIRF) based microarray system that meets these demands and diagnoses sepsis fast and accurate using multiparameter assays. New methods for self-calibration lead to highly accurate, precise and reliable diagnostic reports meeting point-of-care requirements and clinical regulations.

THE SEPSIS CHIP

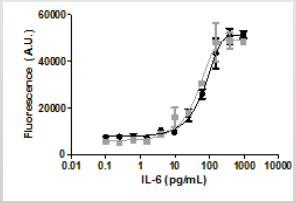
The sepsis chip is a simple and low cost cartridge offering an easy and hygienic handling. The cartridge is consisting of two injection moulded parts being merged with common connection technology (laser welding):

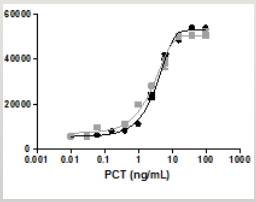
- The microfluidic chip or cover chip with a channel structure manufactured by injection molding and
- The optical chip with prism for light coupling containing the array of protein spots for biomarker detection.

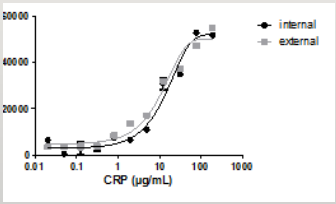
THE BIOMARKERS

Onto the optical chip the capture molecules for biomarker detection are arrayed in triplicate. In addition, guide dots, positive (human IgG) and negative controls (buffer) are spotted.

The biomarkers targeted comprise the inflammation markers C reactive protein (CRP), Interleukin-6 (IL-6) and procalcitonin (PCT), indicating bacterial infection. Optionally, protein biomarker assays for IL-8, IL-10, IL-1ß, VEGF, S-100, neopterin and others can be additionally implemented.







KEY FACTS

- Single-use cartridge for multiplex analysis of CRP, IL-6, and PCT
- Integrated pre-analytics (bilirubin)
- 20 min assay time from sample injection till data read-out
- Works with whole blood, serum and plasma
- Optical read-out based on the TIRF principle