Chip technology for fast serological determination of Salmonella serovars

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Abstract: The present contribution presents a strategy for the serological differentiation of *Salmonella* isolates using a microarray chip with functionalized surfaces for immunoassays. For this aim a microchip was prepared by site-specific immobilization of monoclonal antibodies on microstructured surfaces using spacer-mediated antibody attachment resulting in dense, homogeneous and highly specific spots of antibodies. The immunoassays demonstrated specific recognition of the immobilized immunoglobulines by selected *Salmonella* antigens as determined by fluorescence measurements. It is expected that such miniaturized biofunctional surfaces could be of interest for the development of new solid-phase immunoassay techniques and biosensor techniques due to their potential of high sample throughput and flexibility of analyses as well as of the advantage of saving sample material and analysis time.

keywords: miniaturization, microsystems, serotyp, microchip, immunoassay

Introduction: Microbial contaminations of food by *Salmonella* strains cause a health risks to ultimate consumers as well as economical damages to the food-producing industry. Due to intensified stockbreeding the risk of *Salmonella* infections by contaminated animals kept for meat production has increased.
As a contribution to the development of diagnostic methods on the field of detection and determination of *Salmonella* serovars the development of a chip technology for fast and efficient serological differentiation of *Salmonella* isolates or antibodies in meat or serum was investigated.
The combination of several processes of microfabrication like photolithography and selective etching with specific chemical modification of surfaces, biochemical conjugation and cross-linking allowed the building-up of a carrier chip for detection of biological functional molecules such as *Salmonella* antigens.

Materials and methods: First a suitable method of activation and passivation of the glass surface of the chip was established to avoid unspecific binding processes to the glass substrate during the antigen-antibody interaction. Standard photolithographic techniques were applied to open quadratic windows in the µm range on the substrate surface. These spots were modified with a layer of glycidoxypropyltrimethoxysilane (Lamture et al., 1994). The surrounding areas were chemically passivated by a closed alkylsilane monolayer (Reichert et al., 2000). Further chemical reactions for covalent binding of antibodies and antigens, respectively, were performed on the chips using the epoxy function of the active micro areas. Thus, microarrays i.e. two-dimensional patterns consisting of surface immobilized molecules of defined molecular species were formed.

Results & Discussion: Beside different serovars of *Salmonella* Thyphimurium and of *Salmonella* Choleraesuis various negative control serums and control antigens were included in the investigations to develop and to validate the approach as a model system for serological differentiation of isolates of the species *S. enterica*.
The interactions of antibodies and antigens were monitored by direct and indirect detection using fluorochrome-labeled reagents. The spots were examined using a fluorescence microscope with suitable filters and equipped with a camera. Fluorescence allows the detection of lower density of molecules immobilized at the activated spots in comparison to common agglutination assays, where much higher quantities of antibodies and antigens are needed.
This chip array device will allow a fast serological determination of different serovars of *S. enterica* with the same specificity and reproducibility as the slide agglutination technique. Additional advantages of the microarray chip over the conventionally used immunoadsorption or agglutination assays are shorter analysis time due to shorter diffusion ways for interactions on the chip surface, saving of material for analysis, increase of sample throughput, and the possibility of a further increase of efficiency by automation of analysis and evaluation on the basis of chip readers equipped with suited software.

Conclusion: There are certain advantages in reducing the size of arrays for immunodiagnostic in terms of the quantity of immobilized capture molecule and of sample required for analysis. The high concentration of sample molecule in a small volume should increase the speed at which targets bind to its complementary partner thereby decreasing incubation times. The use of microarrays will have the effect of reducing the storage and packaging facilities required and the size of the instrumentation required for analysis of the arrays.

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References:

Lamture, J. B., Beattie, K. L., Burke, B. E., Eggers, M. D., Ehlich, D. J., Fowler, R., Hollis, M. A., Kosicki, B. B., Reich, R. K., Smith, S. R., Varma, R. S. and Hogan, M. E. (1994): Direct detection of nucleic acid hybridization on the surface of a charge coupled device. *Nucleic Acids Research,* **22,** 2121-2125.

Reichert, J., Csaki, A., Köhler, J. M. and Fritzsche, W. (2000): Chip-based optical detection of DNA hybridization by means of nanobead labeling. *Analytical Chemistry,* **72,** 6025-6029.