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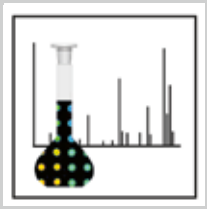

Highlights of Analytical Sciences in Switzerland

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A Lipopolysaccharide Microarray for Analysis of Human Antibodies

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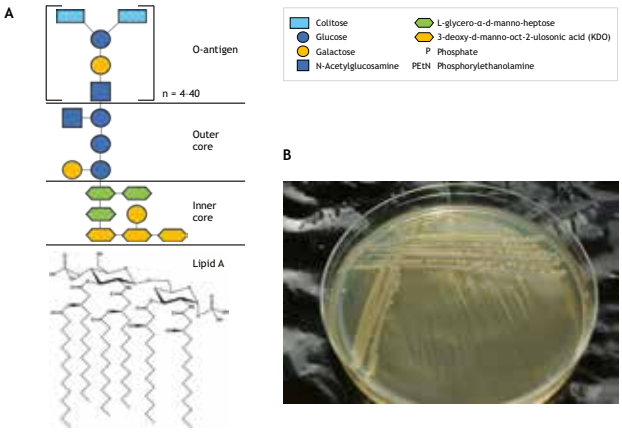
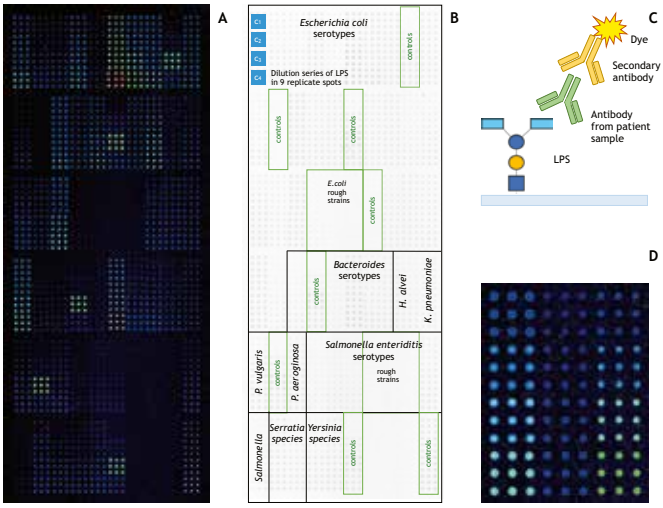
During a lifetime, the immune system is challenged by a large number of bacterial infections, but also by the trillions of commensal bacteria that colonize the gastrointestinal tract. Protective antibodies are produced upon contact with bacterial antigens which specifically bind and control bacterial expansion. The surfaces of Gram-negative bacteria are almost entirely covered with lipopolysaccharides (LPS), providing membrane integrity and stability. Exposed on the cell surface, LPS interact with the surroundings and protect the bacterium from environmental threats, and are known to induce strong immune reactions. The outermost part of the LPS, the O-antigen, is highly diverse structurally and in sugar composition; the basis for bacterial serotyping. About 176 O-antigens are known for *Escherichia coli* alone.



A Schematic overview of *E. coli* O111 LPS, **B** colonies of *Hafnia alvei*.

The tremendous diversity of O-antigens on bacterial lipopolysaccharides contributes to the generation of a vast repertoire of protective antibodies. Analysis of antibody reactivity to LPS in human samples such as blood or breast milk provide an indication about infection history but also confirm the presence of protective antibodies. Microarrays displaying a library of LPS are ideally suited for this kind of analyses. The limiting factor of LPS microarrays is the availability of probes that must be extracted from bacteria in an elaborate process since only a few are commercially available.



A Scan of a microarray incubated with blood plasma, **B** LPS spotting scheme, **C** scheme of a sandwich assay, **D** enlarged part of the array visualizing spot morphology (spotted volume: 1.5 nL).

From the analytical point of view, the limit of detection of a microarray is determined by the affinity of the probe–analyte pair at the surface which is lower compared with solution. The quality of analysis critically depends on spot morphology, *i.e.* the homogeneity of probe distribution within the spot that determines the variability of the fluorescence signal as an essential part of the experimental error. For microarray production, we optimised spotting conditions, buffer composition, pH and ionic strength, and immobilisation conditions for 50 LPS. Array processing conditions were optimised regarding surface blocking (8 h, 23 °C), incubation time and temperature (16 h, 10 °C), including the subsequent reaction with a secondary fluorescence labelled antibody (1 h, 23 °C).

LPS microarrays are convenient tools to study bacterial antigen–antibody interactions and past exposure of an individual to pathogenic and commensal bacteria.

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