Measuring *S. aureus* exotoxin concentrations using protein microarrays with antibodies generated by phage display

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**Objectives:** Objective of the study was to develop a multiplex microarray based system for the fast and quantitative measurement of staphylococcal toxins.

**Methods:** Purified native or over-expressed staphylococcal enterotoxins A and B, toxic shock syndrome toxin, staphylococcal haemolysins alpha and beta, staphylokinase and Panton-Valentine leukocidin (PVL, F-component) were used to generate different specific sets of monoclonal antibodies via phage display. These antibodies were purified after over-expression in *E. coli*, characterised initially by ELISA and spotted in different dilutions in microtiterstrip-mounted protein microarrays.

**Results:** For each toxin, all possible combinations of capture and detection antibodies were tested with microarrays using different protocols and antigen concentrations in order to find the most specific and sensitive antibody combination as well as the fastest possible protocol. These arrays together with a specifically designed software algorithm allow one to measure concentrations of single or multiple staphylococcal toxins in culture supernatants after calibration using recombinant and native toxins.

**Conclusion:** The simultaneous measurement of staphylococcal toxins allows studying the interference between different toxin genes as well as clonal complex-specific variations and differences in toxin gene regulation. These issues and, e.g., a potential clinical significance of hyper-producing strains warrant further study.

**Introduction:**
*S. aureus* harbours a variety of toxin genes which mainly interfere with host defences. This includes leukocidins and superantigens. Some of them apparently have a massive impact on the course of infection. For instance, Panton-Valentine leukocidin (PV) is involved in the pathogenesis of recurring/chronic skin- and soft tissue infections and necrotising pneumonia. The Toxic Shock Syndrome Toxin (TSS) might cause a life-threatening condition, known as Toxic Shock Syndrome (TSS), by a massive, unspecific activation of T-cells. Enterotoxins are frequently involved in food poisoning, but also might cause TSS. While different approaches for the detection of toxin genes are commonly used, the detection of the toxins themselves, in culture supernatants or in patient’s specimens, is currently hardly possible under conditions of a clinical laboratory.

Objective of the study was therefore to develop a multiplex microarray based system for the fast and quantitative measurement of staphylococcal toxins.

**Results:** For each toxin, all possible combinations of capture and detection antibodies were tested with microarrays using different protocols and antigen concentrations in order to find the most specific and sensitive antibody combination as well as the fastest possible protocol. These arrays together with a specifically designed software algorithm allow to measure concentrations of single or multiple staphylococcal toxins in culture supernatants after calibration using recombinant and native toxins.

**Methods:** Purified native or over-expressed staphylococcal enterotoxins A and B, toxic shock syndrome toxin, staphylococcal haemolysins alpha and beta, staphylokinase and Panton-Valentine leukocidin (PVL, F-component) to generate different specific sets of monoclonal antibodies via phage display. Following immunisation of mice, mRNA from B-cells was isolated and amplified. Resulting cDNA, specific for the antigen-binding parts of antibodies, was ligated into bacteriophages and then transformed into *E. coli*.

Antibodies were purified, characterised initially by ELISA and spotted in different dilutions in microtiterstrip-mounted protein microarrays. These were incubated with solutions containing toxins (such as culture supernatants) and then with labelled antibodies. Positive reactions were visualised by horseradish-peroxidase-triggered dye precipitation. Finally, images were taken and analysed using dedicated software (Figure 1).

In a first series of experiments, the expression of PVL by *S. aureus* isolates under uniform culture conditions was targeted showing a correlation between clonal complex affiliation and exotoxin yield with measured PVL concentrations ranging up to 12,000 ng/mL after 18 hrs incubation in KatoKoda medium, as shown in Figure 2A, USA300 and ST93 isolates (including the Queensland clone, ST93-MRSA-IV) reached the highest concentrations of PVL. Interestingly, the PVL yield was very low in isolates which harboured both, *lukF/PV* and *tst1* (average PVL, F-component, of 228 ng/mL in six CC30 isolates and not detectable in two ST942 isolates). Ten *tst1*-positive *S. aureus* isolates yielded TSST concentrations around 200 ng/mL, with a single isolate of ST34-MRSA reaching 1,000 ng/mL.

Similar experiments for other toxins and their combinations are currently underway.

**Conclusions:** The simultaneous, quantitative measurement of staphylococcal toxins in culture supernatants allows studying clonal complex-specific variations in toxin production, differences in toxin gene regulation, effects of antibiotic compounds on toxin secretion or a possible interference between different toxin genes. All these issues as well as, e.g., a potential clinical significance of hyper-producing strains warrant further study.

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