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Development and validation of immuno-based detection methods for Staphylococcal Enterotoxins of *S. aureus* (SEA – SEI)

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Key words
Staphylococcal Enterotoxins, *S. aureus*, foodborne outbreak investigation, multiplex suspension array

Aim of the study
The aim of this work was to develop and validate a highly sensitive and specific multiplex suspension array, based on monoclonal antibodies (mAbs), that recognize a broad range of staphylococcal enterotoxins (SEA, SEB, SEC, SED, SEE, SEG, SEH and SEI) and their protein variants. Most diagnostic tests for the detection of staphylococcal enterotoxins (SE) are based on the detection of the corresponding *se*-genes. Since the heat-stable toxins exert their toxic effect in the absence of the bacterium, DNA detection can be misleading. While DNA detection also has its merits, it displays the potential toxin production on the genetic level, but explicitly not the expression of SEs and their quantity in an outbreak situation. On the protein level, various commercially available methods exist for five SEs (SEA to SEE), with limited sensitivity and specificity. With the developed multiplex suspension array, we aimed at a method for the simultaneous detection, differentiation and quantification of eight SEs in various food matrices.

Material and methods
MAbs against SEA-SEI were generated by classical hybridoma fusion or by recombinant techniques. For comprehensive antibody characterization, surface plasmon resonance spectroscopy and immunological methods were applied. Carefully selected SE-antibodies were implemented in single sandwich-ELISAs and a multiplex suspension array based on the Luminex® technology. Validation of both procedures was performed by following an international recommendation by partners of the EuroBioTox project (https://www.eurobiotox.eu) and included assay performances on 5 days by the same operator and various predefined technical and biological replicates. Recovery rates of spiked SEA to SEI were further determined in common food matrices (raw milk, raw milk cheese and smoked pork paté). To ensure detection coverage of various SE subtypes, 144 whole genome sequenced *S. aureus* strains with a plethora of *se* variants identified on the genetic level were provided by partner institutions and allowed the comparison between theoretical toxin synthesis potential and the actual SEA to SEI detection in their supernatants by the developed immunoassays.

Results and significance
Within the project, a total of 19 mAbs detecting SED, SEH and SEI were newly generated and characterized in detail at the RKI. Classical sandwich-ELISA formats were established for the specific single detection of SED, SEH and SEI, including their protein variants. With additional use of in-house generated mAbs from previous projects (mAbs against SEA, SEB, SEC, SEE and SEG), eight single sandwich-ELISAs and a multiplex suspension immunoarray for the detection of SEA, SEB, SEC, SED, SEE, SEG, SEH and SEI were established. Comprehensive tests confirmed the specificity of the mAbs and the assays. All immunoassays were further analyzed for their recovery rates of spiked toxins in common outbreak-related food matrices (raw milk, raw milk cheese and smoked pork paté), which were processed according to DIN EN ISO 19020. According to the current state of knowledge, both immunoassays – single sandwich-ELISA and the multiplex suspension immunoarray –

showed outstanding characteristics in terms of specificity and sensitivity. The detection limits of all established ELISAs and the multiplex assay were in the low pg/mL range and all immuno-assays detected their native target toxins and their protein variants from bacterial supernatants. For this purpose, 144 whole-genome sequenced *S. aureus* strains were selected for the highest possible SE subtype variance and subsequently their culture supernatants were analysed by immunoassays. Both, the single sandwich-ELISA and the multiplex suspension immunoarray, were able to detect and differentiate all variants of provided SEs in supernatants with an assay accuracy of 90 – 100% and 80 – 100%, for the sandwich-ELISAs and the multiplex suspension array, respectively.
In addition, the protein A problem caused by its affinity towards specific murine immunoglobulin subclasses, leading to high background levels and thus to unreadable results in immunoassays, was solved: The technical solution found was based on the generation of recombinant murine IgG1-antibodies with low affinity for protein A, replacing SE-specific mAbs of different subclasses, and using them in the immunoassays.
All in all, all project aims were successfully reached, and parts of the research undertaken went beyond the originally planned work. Notably, the multiplex suspension immunoarray developed in this work allows for the first time the simultaneous detection, differentiation and quantification of multiple SEs from minimal sample volumes.

Publications, posters and presentations

Dettmann, P. (2020) Detektion, Differenzierung und Quantifizierung von *Staphylococcus aureus* Enterotoxinen. ICAR3R-Symposium/ Aktuelle Debatte in der 3R-Forschung, online, 01 October 2020.

Dettmann, P. (2020) Detection, Differentiation and Quantification of Staphylococcal Enterotoxins (Methodical). RoKoCon2020 – Outbreak Response, online, 30 October 2020.

Dettmann, P. (2021) Multiplex Assay for Detection, Differentiation and Quantification of Staphylococcal Enterotoxins. Zoonoses 2021 – International Symposium on Zoonoses Research, online, 13 – 15 October 2021.

Dettmann, P. (2022) Rational Design of Highly Sensitive Immunoassays for Detection of Staphylococcal Enterotoxin B. RoKoCon 2022 – Robert Koch Doctoral Students Symposium, Robert Koch Institute, Berlin, 29 – 30 September 2022.

Dettmann, P. (2023) Simultaneous Detection, Differentiation and Quantification of Eight Staphylococcal Enterotoxins in a Multiplex Suspension Assay. 8th German Pharm-Tox Summit, University Ulm, 07 – 09 March 2023.

Dettmann, P. (2023) Pitch Promotion – Detektion, Differenzierung und Quantifizierung von Staphylokokken Enterotoxinen (SEA – SEI) mit einem Multiplex-Suspensionsarray. Internes Seminar – Forschung der nächsten Generation am RKI, online, 03 May 2023.

Awards

Dettmann, P. (2021) Poster award, Multiplex Assay for Detection, Differentiation and Quantification of Staphylococcal Enterotoxins. Zoonoses 2021 – International Symposium on Zoonoses Research, online, 13 – 15 October 2021.

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