

Multiplex analysis by mass spectrometry: a strong and alternative tool for the detection of staphylococcal enterotoxins involved in food poisoning outbreaks

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Staphylococcal food poisoning outbreaks (SFPO) are caused by the ingestion of food contaminated with staphylococcal enterotoxins (SEs) produced by strains of coagulase positive Staphylococci, and their notification is mandatory. The suspected food is sampled and submitted to several type of analysis allowing the enumeration of the bacteria, detection of their enterotoxins genes and the detection of the enterotoxin produced in the food as the ingestion of is last one is responsible of the disease.

To date, 33 SEs are described in the literature but only 5 SEs (SEA to SEE) can be routinely detectable *via* commercially available immunoassays (e. g. EN ISO 19020). Even ELISA methods are the most commonly used and considered as the most sensitive, false positive results can be obtained in complex matrices as reported in the EN ISO 19020 method. Thus, the competent authority recommends the use of a confirmatory analysis based on a different principle.

In this work, we present an LC-MS method developed under our European Union Reference Laboratory mandate for detection of 8 SEs in food. SEs are extracted from food matrices by immuno-capture (IC), and then submitted to trypsin digestion before analysis by LC-HRMS/MS. For each type of SEs, between 3 and 8 proteotypic peptides were optimized using sequences (including sequence variants) obtained from more than 500 strains from different SFPO occurred in Europe. [1]

This LC-HRMS/MS method was implemented during the investigation of several SFPO occurred in France and Europe (Italy) between 2016 and 2022. The data obtained by mass spectrometry were compared to those from EILISA, PCR and genomic analyses. A correlation was observed between LC-MS, ELISA and molecular typing methods. The high specificity and multiplex analysis of mass spectrometry are the main advantage over commonly used ELISA methods.

References

1. Lefebvre, D.; Blanco-Valle, K.; Feraudet-Tarisse, C.; Merda, D.; Simon, S.; Fenaille, F.; Hennekine, J.-A.; Nia, Y.; Becher, F. J. Agric. Food Chem, **69**, 2603–2610 (2021).