Interlaboratory proficiency tests for the detection of staphylococcal enterotoxin type A in food

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Significance and Impact of the Study: Staphylococcal enterotoxins (SEs) can cause food poisoning even at very low doses. We organized three interlaboratory proficiency tests (ILPTs) to assess the competence of the official laboratories in Germany in detecting SE type A in food. To improve their competence, we identified the main factors contributing to the overall low sensitivity detected in 2013. The improvements in 2014 and the very satisfactory outcome of the 2018 ILPT, where all performance criteria were above 90%, reflect the joint efforts of our National Reference Laboratory and the official food control laboratories in Germany to successfully implement and improve SE detection in food.

Keywords
coagulase-positive staphylococci (CPS), dairy products, food safety, food control, foodborne intoxication, immunoassay, staphylococcal food poisoning, *Staphylococcus aureus*.

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Abstract
Staphylococcal enterotoxins (SEs) are among the leading causes of food intoxications, affecting consumer health even in nanogram (ng) amounts. In the European Union, certain food safety criteria are specified, including the absence of SEs in cheeses, milk powder and whey powder. Until 2019, the analytical reference method used was the European Screening Method, which was replaced by EN ISO 19020. For the official laboratories involved in food control, the German Reference Laboratory for coagulase-positive staphylococci including *Staphylococcus aureus* organized three interlaboratory proficiency tests (ILPTs) to detect SE type A in food during the years 2013–2018. The selected food products (cream cheese and vanilla pudding) were successfully tested beforehand with regard to easy handling, homogeneity and stability of the added toxin. In 2013, ILPT participants overall were not competent in detecting SE type A in food. The following factors were identified to improve the performance: (i) concentration of sample extract using dialysis; (ii) selection of a sensitive detection kit; and (iii) proper sample handling. By taking these factors into account and instructing and training the laboratories, their competence greatly improved. In 2018, all performance criteria (specificity, sensitivity and accuracy) were >90%, even at very low concentrations of SE type A of approximately 0.01 ng g⁻¹ food.

Introduction
Staphylococcal enterotoxins (SEs) represent a major cause of food intoxications and can be formed in food by coagulase-positive staphylococci (CPS), particularly *Staphylococcus aureus*, under a wide range of conditions (Schelin *et al*. 2011). Primary sources of SE-producing bacteria include food-producing animals and human carriers, and staphylococcal food poisoning outbreaks have been particularly associated with dairy products and ready-to-eat foods (Hennekinne *et al*. 2012). It was estimated that even very low amounts of approximately 20–100 ng SE are enough to cause an outbreak (see, for example, Asao *et al*. 2003; Ostyn *et al*. 2010). To date, at least 24 types of SEs and SE-like proteins have been identified, which are named with consecutive letters (Fisher...
et al. 2018). The five classical SE types A to E (referred to as SEA–SEE), and SEA in particular, have been frequently associated with food poisoning outbreaks; however, they are also the most commonly studied and analytical detection methods are most available for them, including commercial immunoassays (Hennekinne et al. 2012; Fetsch and Johler 2018). In addition, other SEs, such as SEH, may also be involved in food poisoning outbreaks (Sche- lin et al. 2011; Fisher et al. 2018).

In the European Union (EU), mandatory notification of food-borne outbreaks has been required since 2005. Commission Regulation (EC) No. 2073/2005 (EU 2005) established microbiological criteria for food safety, including undetectable levels of SEs in cheeses, milk powder and whey powder (placed on the market) when analysed in a 25-g sample by the analytical reference method. In addition, process hygiene criteria have been defined for CPS in these foodstuffs, and batches must be tested for SEs if the CPS count exceeds $10^5$ colony forming units per gram (EU 2005).

Detection of SEs in food is routinely done using a qualitative approach. Since very small SE amounts can trigger food poisoning, a sensitive method for SE detection—including efficient extraction from food products—is required to ensure food safety. Until 2019, the analytical reference method for SE surveillance in the EU was the European Screening Method (ESM) of the EU Reference Laboratory (EU-RL) for CPS. The reference method targets the five classical types SEA–SEE and utilizes commercially available sensitive immunological assays. Based on the latest ESM version, ESM v.5 (EU-RL for CPS 2010), an international standard was established: ISO 19020:2017 (ISO 2017). In February 2019, the EN ISO 19020 replaced the ESM v.5 in the EU Food Law as the official reference method for the detection of SEs (EU 2019). Both methods aim at a qualitative detection of SEs. However, the sample processing is relatively complex and requires practice. Suitable reference material for implementation and verification of the methods in laboratories was scarce. In Germany, the official food control is undertaken by the official laboratories of the federal states. The National Reference Laboratories (NRLs) support the federal state laboratories in their tasks, for example by providing reference material, method recommendations, training and confirmation analyses. They also organize interlaboratory proficiency tests (ILPTs) in order to evaluate the ability of laboratories to perform their official duties.

In the period from 2013–2018, the National Reference Laboratory for coagulase-positive staphylococci including *Staphylococcus aureus*, based in Germany (hereafter referred to as the German NRL for CPS), organized three ILPTs for the detection of SEA in dairy products. The ILPTs targeted the laboratories involved in official food control in Germany and aimed at assessing their performance in detecting SE in food in order to protect consumers’ health.

### Results and discussion

Between 2013 and 2018, a total of three ILPTs for the qualitative detection of SEA in food were organized by the German NRL for CPS (Fetsch et al. 2016). SEA is the SE type that was by far most frequently associated with food poisoning outbreaks (Fisher et al. 2018). After conducting two ILPTs with cream cheese, participants were challenged with another dairy product, vanilla pudding, in 2018 (Table 1), since staphylococcal food poisoning outbreaks have also been associated with dairy-based deserts (Hennekinne et al. 2012; Fetsch et al. 2014; Ercoli et al. 2017). Both food products were found to be suitable with regard to easy handling, homogeneity and stability of SEA over the duration of the ILPTs (see also Table S1). Sufficient stability in the food was given both when SEA was dissolved in phosphate-buffered saline (in 2013 and 2014) and in water (in 2018) before it was added to the food. Three levels of contamination were used in the ILPTs: non-spiked (blank), low concentration of SEA

### Table 1 Overview of the three ILPTs for the detection of staphylococcal enterotoxin A (SEA)

| ILPT   | Food product   | Samples* | Approx. SEA concentration in the samples | No. of participating laboratories† |
|--------|----------------|----------|-----------------------------------------|-----------------------------------|
|        |                |          | Blank | SEA low | SEA high |                                  |
| 2013   | Cream cheese   | 1× blank; 2× SEA low; 2× SEA high | n.d. | 0.011–0.013 ng g$^{-1}$ | 0.046–0.050 ng g$^{-1}$ | 14 |
| 2014   | Cream cheese   | 1× blank; 2× SEA low; 2× SEA high | n.d. | 0.008–0.010 ng g$^{-1}$ | 0.041–0.044 ng g$^{-1}$ | 9  |
| 2018   | Vanilla pudding| 1× blank; 2× SEA low; 2× SEA high | n.d. | 0.009–0.011 ng g$^{-1}$ | 0.030–0.049 ng g$^{-1}$ | 19 |

*Each 25 g ± 0.1 g.
†n.d.: not detected. For further information on the SE quantification in the ILPT samples, see Table S2.
‡Participants in all three ILPTs included the EU-RL for CPS, and in 2018, the French NRL for CPS and the German NRL for CPS as well. The number of participants only included laboratories considered for data assessment.
(SEA low) and higher concentration of SEA (SEA high) (Table 1).

In 2013 and 2014, ILPT participants were recommended to use the ESM v.5 and were also given the opportunity to test up to two methods for sample preparation. In 2018, ILPT participants were recommended to use either the ESM v.5, which was still the official reference method at that time (EU 2018), or the (DIN) EN ISO 19020 (ISO 2017) standard. The two recommended methods have only minor technical differences, and both include pH adjustments (acidification and neutralization) during the sample extraction and an extract concentration step by dialysis. An SE detection kit is used for the detection process. According to the ESM v.5, the detection must be done using one of the following commercial immunoenzymatic kits: Vidas SET2 (bioMérieux), Ridascreen SET Total (r-biopharm). The two kits allow sensitive detection of SEA–SEE, without distinguishing between these five SE types. The ISO 19020 method does not specify a particular SE detection kit, but defines performance criteria that the kit of choice should meet: The limit of detection, LOD_{50}, should be <0.06 ng g^{-1}; the sensitivity and specificity should be higher than 90% (ISO 2017). In the three ILPTs, participants were allowed to submit results obtained with up to two different detection kits for each sample preparation method. Overviews of the methods and detection kits used in the ILPTs can be found in Tables 2 and 3.

The number of ILPT participants ranged from 9 to 19 (Table 1). In addition to German laboratories, the EU-RL for CPS, and in 2018, the French NRL for CPS (both located at the French Agency for Food, Environmental and Occupational Health & Safety, ANSES) as well participated in the ILPTs. The individual results from all participants in the three ILPTs are presented in Tables S3–S5, which show all tested method/kit combinations. It must be noted that, particularly in 2013, during the course of implementing and verifying the method, some additional overviews of the methods and detection kits used in the ILPTs can be found in Tables 2 and 3.

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### Table 2 Methods applied by ILPT participants for sample preparation

| Method* | Dialysis of extract | 2013 | 2014 | 2018 |
|---------|---------------------|------|------|------|
|         |                     | No. | %   | No. | %   |
| (DIN) EN ISO 19020:2017 | Yes | –   | –   | 8   | 42.1 |
| ESM v.5 | Yes                 | 7   | 41.2 | 8   | 80.0 |
|         | No                  | 1   | 5.9  | 0   | 0.0  |
| Manual Vidas SET2 | Yes† | 0   | 0.0  | 0   | 0.0  |
|         | No                 | 4   | 23.5 | 1   | 10.0 |
| Manual Ridascreen SET Total | Yes† | 0   | 0.0  | 0   | 0.0  |
|         | No                 | 3   | 17.6 | 0   | 0.0  |
| Manual Ridascreen SET A,B,C,D,E | Yes† | 0   | 0.0  | 0   | 0.0  |
|         | No                 | 2   | 11.8 | 1   | 10.0 |
| Manual Oxoid SET-RPLA | No | 0   | 0.0  | 0   | 0.0  |
| Total no. of method-run tests performed | 17* | 10* | 19 | |
| No. of participating laboratories | 14 | 9 | 19 |

*Manual referred to ESM v.5.
†Manual referred to ESM v.5.

### Table 3 Commercial kits used by ILPT participants for detection of staphylococcal enterotoxins

| Detection kit* | 2013 | 2014 | 2018 |
|---------------|------|------|------|
|               | No. | %   | No. | %   | No. | %   |
| Vidas SET2 (bioMérieux) | 7   | 35.0 | 4   | 28.6 | 8   | 34.8 |
| Ridascreen SET Total (r-biopharm) | 9   | 45.0 | 5   | 35.7 | 10  | 43.5 |
| Ridascreen SET A,B,C,D,E (r-biopharm) | 4   | 20.0 | 4   | 28.6 | 4   | 17.4 |
| SET-RPLA (Oxoid) | 0   | 0.0  | 0   | 0.0  | 1   | 4.3  |
| VIA (TECRA) | 0   | 0.0  | 1   | 7.1  | 0   | 0.0  |
| Total no. of kit-based tests performed | 20* | 14* | 23* |
| No. of participating laboratories | 14 | 9 | 19 |

*For each sample extract, results obtained with up to two different detection kits could be submitted by the participants.
methods were tested in comparison, which participants may have already suspected to be less suitable. However, even taking this assumption into account, the overall performance—particularly sensitivity—of SE detection was concerning in the 2013 ILPT (Table 4; Fig. S1). Even when considering only the best method/detection kit used by each participant, the total sensitivity was only 55%, and was as low as 32% for the low SEA-contaminated samples. The accuracy was only 64%. In 6 of the total of 22 method/kit tests performed in 2013, SE could not be detected in any of the samples, neither in the low nor in the higher contaminated ones (Table S3).

As previously discussed in detail (Fetsch et al. 2016), the main reason for the low sensitivity and low accuracy in the first ILPT was the lack of concentration of the sample extract by dialysis (Table 5). None of the method/kit tests performed in 2013 that were performed without an extract concentration were able to detect all SEA-positive samples. Conversely, all correct results (with 100% specificity, 100% sensitivity and 100% accuracy) were achieved with methods that included an extract concentration by dialysis. However, even when dialysis was performed, the sensitivity for the low-contaminated samples was 61% (Table 5). Five of ten method/kit tests failed to correctly detect all SEA-contaminated samples; when considering only those method/kit tests performed in compliance with the ESM v.5, four of eight failed (Table S3). This indicates that sample handling errors contributed to the poor performance in the 2013 ILPT. The best performance was obtained with the Vidas SET2 kit. This automated test requires a special technical device, but considerably less manual handling than the Ridascreeen kits, which are manual sandwich enzyme immunoassays. Shortcomings in the application of the method are generally particularly evident at low SE concentrations.

The dairy products used for the ILPTs were contaminated to approximately 0.01 to 0.05 ng g⁻¹ (Table 1). These toxin levels are very low, but could still be of toxicological concern, particularly at the higher concentration. The benchmark dose for SEA that is likely to trigger health symptoms in 10% of the exposed population has been estimated to be as low as 6·1 ng (Guillier et al. 2016). Even food with as little as 0·02 ng SEA per g has been associated with a staphylococcal food poisoning outbreak (Denayer et al. 2017). The SEA concentrations in the ILPT samples were above the limit of detection of the ESM v.5 and were in a similar range as the concentrations used by the EU-RL for CPS for their ILPTs (compare Fetsch et al. 2016).

Following the 2013 ILPT, strong efforts were made to improve the competence of the national official laboratories with regard to detecting SEs in food—even at very low levels of contamination. Measures provided by the

| Year | Total SEA low | Total SEA high |
|------|---------------|----------------|
| 2013 | 100% (20/20)  | 94% (95/100)   |
| 2014 | 100% (15/15)  | 95% (95/100)   |
| 2018 | 100% (23/23)  | 94% (94/100)   |

*Sample numbers are shown in brackets. Percentage values are also presented in Fig. S1.
German NRL for CPS included in-depth methodological training for laboratories and provision of SEA-contaminated reference material for method implementation and verification. The efforts undertaken were successful and increased the performance in the subsequent ILPTs (Table 4; Fig. S1). Twelve of the 14 ILPT participants from 2013 also participated in 2014 and/or 2018. In addition, two and five German laboratories participated for the first time in 2014 and 2018, respectively.

In the 2014 ILPT, sensitivity for the low-contaminated samples increased from 32% to 89% (considering one method/kit combination per participating laboratory). In 2014, failures in detecting SEA only occurred in cases where dialysis was not performed or when a detection kit with potentially low sensitivity (here, the TECRA VIA kit) was used (Table 5; Table S4). The TECRA VIA kit showed a lower sensitivity in several food products and has now been withdrawn from the market (ISO 2017).

In 2018, all performance criteria (specificity, sensitivity, accuracy) were higher than 90%—including the sensitivity obtained for the low SEA-contaminated samples (Table 4; Fig. S1). Thus, the overall high performance observed in 2014 was maintained—despite the switch to another dairy product—and the sensitivity for low SEA concentrations was further increased. The misclassification of SEA-contaminated samples by participants in the 2018 IPLT was either due to a handling problem with the first sample or was caused by sample preparations that lacked extract concentration by dialysis in combination with a lower sensitivity detection kit (Oxoid SET-RPLA kit) (Table 5; Table S5). The limit of detection specified by the manufacturer is higher for the SET-RPLA kit compared to the Vidas and Ridascreen kits. However, the Oxoid SET-RPLA kit enables a visual detection of SEA—SED (but not SEE) without the need for photometric measurement.

Eight of the 19 laboratories that participated in the ILPT in 2018 have already applied the (DIN) EN ISO 19020:2017 standard, and successfully in all cases. There are only minor technical differences between the ESM v.5 and the ISO reference methods. Consequently, laboratories that have successfully implemented the ESM v.5 should be able to comply with the (DIN) EN ISO 19020 standard without significant effort. In addition to the two detection kits specified in the ESM v.5 (Vidas SET2 and Ridascreen SET Total), the Ridascreen SET A,B,C,D,E kit (r-biopharm) was also successfully used by participants in the 2014 and 2018 ILPTs to detect both the higher and the very low concentrations of SEA in dairy products.

At the national level, only 8 German laboratories from 6 federal states participated in the 2014 ILPT, whereas 16 German official laboratories from 12 federal states participated in the 2018 ILPT (not counting the German NRL for CPS). As a result, not only the performance but also the dispersion of competence for the detection of SES across the German federal states and among the laboratories involved in official food control has greatly improved. The strong ability of the national official laboratories to

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**Table 5** Sensitivities achieved with different combinations of detection kit and sample preparation method with or without concentration of the sample extract by dialysis*

|                  | 2013    | 2014    | 2018    | Total   |
|------------------|---------|---------|---------|---------|
|                  | Sensitivity |       | Sensitivity |       | Sensitivity |       | Sensitivity |       | Sensitivity |       |
|                  | SEA      | low    | high    | n†      | SEA      | low    | high    | n†      | SEA      | low    | high    | n†      |
| All method/kit combinations with dialysis | 9  61-1% 100% 12  91-7% 100% 22  97-7% 100% 43  90-5% 100% |
| Dialysis, Vidas SET2 | 2  100% 100% 3  100% 100% 8  100% 100% 13  100% 100% |
| Dialysis, Ridascreen SET Total | 5  40% 100% 5  100% 100% 10  95% 100% 20  82-5% 100% |
| Dialysis, Ridascreen SET A,B,C,D,E | 2  75% 100% 3  100% 100% 4  100% 100% 9  94-4% 100% |
| Dialysis, TECRA VIA | – – – 1  0% 100% – – – 1  0% 100% |
| All method/kit combinations without dialysis | 11  0% 45-5% 2  0% 100% – – – 14  0% 50% |
| No dialysis, Vidas SET2 | 5  0% 100% 1  0% 100% – – – 6  0% 100% |
| No dialysis, Ridascreen SET Total | 4  0% 0% – – – 4  0% 0% |
| No dialysis, Ridascreen SET A,B,C,D,E | 2  0% 0% 1  0% 100% – – – 3  0% 33-3% |
| No dialysis, Oxoid SET-RPLA | – – – 1  0% 0% 1  0% 0% |

*The specificity was 100% in all cases.
†n: Number of ILPT participants that applied the method/kit combination (for two replicate samples per contamination level).
detect very low levels of SE (down to approximately 0.01 ng SEA per g food, corresponding to approximately 0.05 ng SEA per ml concentrated sample extract) ensures a high level of food safety.

To conclude, the main critical points for successful detection of SE in food, especially when present at low concentrations that may already pose a health risk, are: (i) dialysis of sample extracts; (ii) use of a sensitive detection kit; and (iii) practical experience of laboratory personnel in handling the sample (e.g. during pH adjustment to not exceed the recommended range of pH and during extract recovery after dialysis). In addition to the ILPTs of the EU-RL and NRLs, certified reference material supports the establishment and verification of the method used in laboratories involved in food control (Zeleny et al. 2016). Further analytical developments are required and are in progress to broaden the spectrum of detectable SE types and limit potential interferences between some food products and detection assays.

Materials and methods

Material and ILPT sample preparation

The food product used for the ILPTs in 2013 and 2014 was cream cheese; vanilla pudding was used for the 2018 ILPT (Table 1). Details of the first ILPT are described in other sources (Fetsch et al. 2016). All food products were purchased at retail and tested for the absence of SEA–SEE as described below. The SEA was obtained from the Staphylococcus aureus reference strain CCUG 47208 (Culture Collection University of Gothenburg, Sweden) after being grown in brain heart infusion broth (Merck, Darmstadt, Germany) at 37°C for 48 h. The culture supernatant was sterile-filtered and stored at –21 ± 3°C as lyophilized aliquots until use. For the three ILPTs, aliquots of one SEA batch were used. Before spiking the ILPT samples, the lyophilized SEA was dissolved and diluted into two different concentrations using either phosphate-buffered saline (for the ILPTs in 2013 and 2014) or ultrapure water (for the 2018 ILPT). In all three ILPTs, the dilution factors were the same and the same volume (500 µl for each 25-g sample) was used for the spiking. Three levels of contamination were used for each ILPT (Table 1), and each participant received five samples: one blank and two samples each of a low and a higher SEA concentration. The ILPT samples were coded with sample numbers and all samples were kept frozen (at –21 ± 3°C) until analysis.

The following safety precautions must be observed when handling SEA: use personal protective equipment; avoid contact with eyes and skin; avoid formation of dust and aerosols; avoid inhalation of dust/aerosols; do not allow SEA to drain into sewers; dispose of waste in a safe way in accordance with local regulations.

Homogeneity and stability of ILPT samples

The homogeneity and stability of the ILPT samples were tested in 2013, 2014 and 2018 with 27, 40 and 42 samples, respectively, using the analytical methodology described in the next subsection. The samples of all three ILPTs met the following criteria (see also Fetsch et al. 2016).

Homogeneity (tested before delivery of samples): All randomly selected blank samples were tested negative for SEA–SEE. All randomly selected SEA-contaminated samples were tested SEA-positive, and for each contamination level, the relative standard deviation was below 15% (mostly lower than 10%).

Stability (tested during and after the end of the investigation period): All randomly selected blank samples were tested negative for SEA–SEE. All randomly selected SEA-contaminated samples were tested SEA-positive, and for each contamination level, the standard deviation relative to the mean test value obtained in the homogeneity study was below 15% (mostly lower than 10%).

Analytical methods used for ILPT organization

Qualitative detection of SEA–SEE in the non-spiked food products (prior to ILPT sample preparation) and in the ILPT samples (during homogeneity and stability testing) was performed in the German NRL for CPS in 2013 and 2014 according to the ESM v.5 method (EU-RL for CPS 2010) and in 2018 pursuant to the DIN EN ISO 19020:2017-09 standard (ISO 2017). Sample extracts were concentrated by dialysis as described in both reference methods using a Spectra/Por 1 MWCO 6–8 kDa membrane (Spectrum Laboratories, Rancho Dominguez, Los Angeles, CA). The concentrated extracts were recovered from the membrane using phosphate-buffered saline. The detection of SEA–SEE in each concentrated sample extract was determined using both the Vidas SET2 (bioMérieux, Marcy l’Etoile, France) and the Ridascreen SET Total (r-biopharm, Darmstadt, Germany) kits in accordance with the manufacturers’ instructions. Samples with values above the cut-off of the detection kit were rated as positive for SEA–SEE (Table S1).

The quantitative determination of SEA, SEB, SEC and SED in the ILPT samples (using two replicate samples each with low and higher SEA contamination and one blank sample) was carried out at the EU-RL for CPS utilizing in-house enzyme-linked immunosorbent assays (ELISAs). The ELISAs were based on commercially available antibodies and SE standards, all obtained from Toxin
Technology (Sarasota, FL). For SEA, SEC and SED quantification, double-sandwich ELISA types were used, whereas a single-sandwich type was used for SEB. The sample extraction and concentration were performed according to the ESM v.5 method. Further details on SE quantification are provided with Table S2.

ILPT participants and sample delivery

The ILPTs were particularly aimed at laboratories involved in official food control in Germany. In addition to these official laboratories of the German federal states, a laboratory of the German Federal Armed Forces and the German NRL for CPS took part in 2018. Furthermore, the EU-RL for CPS located at the ANSES in France participated in all three ILPTs, and in 2018, the French NRL for CPS (also located at the ANSES) took part as well.

The ILPT sample sets were delivered frozen on dry ice. The German laboratories received the samples the following day; the French laboratories within 48 h. All participants confirmed receipt of the samples in undamaged and frozen condition.

Choice of analytical methods for ILPT participation and test period

Participants in the ILPTs were free to choose an analytical method, but in 2013 and 2014, it was recommended to use the ESM v.5 (EU-RL for CPS 2010) and in 2018 to use either the (DIN EN) ISO 19020:2017 (ISO 2017) or the ESM v.5 standard. In 2013 and 2014, participants were allowed to test up to two methods for sample preparation (upon request with parallel sample sets) as well as multiple SE detection kits. In 2018, each participant had to choose one sample preparation method, but results obtained with a second detection kit could also be submitted. Samples needed to be analysed by ILPT participants within approximately 3 weeks (2013 ILPT), 4 weeks (2018 ILPT) or 5 weeks (2014 ILPT) after sample delivery.

Performance criteria

The detection of SEs in food according to Commission Regulation (EC) No. 2073/2005 and in the context of our ILPTs constitutes a qualitative analysis. To evaluate the performance of SE detection in food, we determined the specificity, sensitivity and accuracy of the results (Baratloo et al. 2015; Fetsch et al. 2016; ISO 2017). Specificity here describes the ability to obtain a negative result for a sample that does not contain SEA–SEE. It was calculated as follows:

\[
\text{Specificity}(\%) = \frac{\text{No. of samples correctly identified as negative}}{\text{No. of blanks tested}} \times 100.
\]

In our case, sensitivity describes the ability to obtain a positive result for a sample that does contain SEA. It was calculated as follows:

\[
\text{Sensitivity}(\%) = \frac{\text{No. of samples correctly identified as positive}}{\text{No. of positive samples tested}} \times 100.
\]

Here, accuracy describes the ability to obtain a correct result for samples that do not contain SEA–SEE and for samples that do contain SEA. It was calculated as follows:

\[
\text{Accuracy}(\%) = \frac{\text{No. of samples correctly identified to be positive or negative}}{\text{Total no. of samples tested}} \times 100.
\]

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Conflict of Interest

The authors declare that they have no conflict of interest.

Author contributions

S. Schaarschmidt: conception and design of the study; analysis of data; drafting and revising the manuscript. D. Leeser-Boek & K. Drache: data acquisition. Y. Nia: data acquisition; analysis of data. G. Krause & A. Fetsch: conception and design of the study; analysis of data. All authors have approved the final submitted version.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Illustration of the performance of the ILPT participants over years.

Table S1 Variation in the samples analysed at the German NRL for CPS over the entire duration of the ILPTs.

Table S2 Staphylococcal enterotoxin contents in the ILPT samples.

Table S3 Individual results of the 2013 ILPT (Fetsch et al. 2016), including all method/kit combinations tested.

Table S4 Individual results of the 2014 ILPT.

Table S5 Individual results of the 2018 ILPT.