भारतीय मानक

Indian Standard

IS 18355 : 2023
ISO 19020 : 2017

खाद्य श्रृंखला की सूक्ष्मजैविकी — खाद्य पदार्थों में स्टेफाइलोकोकल एंटरोटॉक्सिन का इम्यूनोएंजाइमेटिक पद्धति द्वारा पता लगाने की क्षैतिज विधि

Microbiology of the Food Chain — Horizontal Method for the Immunoenzymatic Detection of Staphylococcal Enterotoxins in Foodstuffs

ICS 07.100.30

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Food Microbiology Sectional Committee, FAD 31

NATIONAL FOREWORD

This Indian Standard which is identical to ISO 19020 : 2017 'Microbiology of the food chain — Horizontal method for the immunoenzymatic detection of staphylococcal enterotoxins in foodstuffs' issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standards on the recommendation of the Food Microbiology Sectional Committee and approval of the Food and Agriculture Division Council.

The text of ISO Standard has been approved as suitable for publication as an Indian Standard without deviations. Certain conventions are, however, not identical to those used in Indian Standards. Attention is particularly drawn to the following:

a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'; and

b) Comma (,) has been used as a decimal marker while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

In this adopted standard, reference appears to the following International Standard for which Indian Standard also exists. The corresponding Indian Standard which is to be substituted in its place is listed below along with its degree of equivalence for the edition indicated:

International Standard	*Corresponding Indian Standard*	*Degree of Equivalence*
ISO 7218 Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations	IS 16122 : 2013/ISO 7218 : 2007 Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations	Identical

The technical Committee has reviewed the provisions of the following International Standard referred in this adopted standard and has decided that it is acceptable for use in conjunction with this standard:

International Standard	*Title*
ISO 3696	Water for analytical laboratory use — Specification and test methods

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 2022 'Rules for rounding off numerical values (*second revision*)'.

Contents

Page

Introduction

Staphylococcal enterotoxins (SEs) are proteins that can be produced in foods, by certain strains of the coagulase positive staphylococci (CPS), mainly *Staphylococcus aureus*. These SEs are heat and acid stable toxins that cause nausea, vomiting, abdominal pain and diarrhoea when ingested. Due to their stability SEs might still be present even when coagulase positive staphylococci cannot be detected. SEs consist of a family of more than 20 structurally-related globular monomeric proteins with molecular weights of 19 kDa to 30 kDa.[1] These proteins are relatively stable under changing environmental conditions, such as heat treatment, freezing and change in pH; moreover, they are resistant to proteolytic digestion. Typically, and depending on the sensitivity of affected individuals, nanogram (ng) amounts of enterotoxin can cause intoxication with the symptoms described above. Due to the influence of SEs on human health, the European Union has adopted legislation in order to increase consumer protection by defining microbiological criteria for foodstuffs, such as CPS enumeration and detection of SEs.[2]

Several methods have been developed for the detection and/or quantification of SEs. Some of these methods are based on enzyme immunoassay (EIA). Other methods are based on the chemical analysis using liquid chromatography with tandem mass spectrometry (LC-MS/MS) for the detection and quantification of SEs. As these latter methods are currently under development, EIA methods have been chosen as the starting point for standardization of a detection method for SEs.

The aim is to detect SEs using commercially available test kits. This document describes the protocol for the extraction of SEs from food samples. Moreover, criteria for the performance of the kits have been evaluated on five types of food matrices before use based on the criteria given in this document.

Response rates of different staphylococcal food poisoning outbreaks were modelled as a function of ingested doses.[3] For this purpose, data from the literature as well as data from the European Union Reference Laboratory for CPS were used.

The United States Environmental Protection Agency (US EPA) benchmark dose methodology was applied to this data set and helped to establish the benchmark dose (BMD).[4] The BMD is defined as the dose of a hazard (staphylococcal enterotoxin) likely to trigger health symptoms in a given percentage of the exposed population. The BMD lower limit (BMDL) is the lower 95 % (or 90 %) confidence interval of the BMD. This value was used to set up the acceptable value for the limit of detection 50 (LOD_{50}) of the various commercially available SE detection kits.

Indian Standard

MICROBIOLOGY OF THE FOOD CHAIN — HORIZONTAL METHOD FOR THE IMMUNOENZYMATIC DETECTION OF STAPHYLOCOCCAL ENTEROTOXINS IN FOODSTUFFS

1 Scope

This document specifies a screening method for the detection of staphylococcal enterotoxins SEA, SEB, SECs, SED and SEE in foodstuffs. It consists of two main steps: a) extraction followed by a concentration based on dialysis principle; and b) an immunoenzymatic detection using commercially available detection kits.

This document is applicable to the screening of staphylococcal enterotoxins SEA to SEE in products intended for human consumption.

Other staphylococcal enterotoxins such as types SEG, SEH, SEI, SER, SES and SET can also cause illness. Due to the lack of commercially available detection kits, this document is applicable only to types SEA to SEE, but may apply to other types of toxins, subject to validation of the method.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

3.1
staphylococcal enterotoxin A, B, C, D, E
SEA, SEB, SEC, SED, SEE
exoprotein SEA, SEB, SEC, SED and SEE produced by enterotoxigenic strains of coagulase positive staphylococci, mainly *Staphylococcus aureus* with a molecular weight ranging from 19 kDa to 30 kDa

3.2
specificity
SP
number of samples found to be negative divided by the total number of blank samples tested

3.3
sensitivity
SE
number of samples found to be positive divided by the total number of samples tested at a given level of contamination

3.4
limit of detection 50
LOD_{50}
concentration (ng SE/g) for which the probability of detection is 50 %

3.5
benchmark dose
BMD
dose of a hazard (e.g. staphylococcal enterotoxin) likely to trigger health symptoms in a given percentage of the exposed population

4 Principle

This document specifies a method for the detection of staphylococcal enterotoxins (SEA to SEE) in all foodstuffs, consisting of two main steps: a) extraction followed by a concentration based on dialysis principle; and b) an immunoenzymatic detection using commercially available detection kits.

5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

5.1 Distilled or **demineralized water** or **water of equivalent quality** according to ISO 3696.

5.2 Hydrochloric acid (e.g. concentrations 5N, 1N or other dilutions).

5.3 Sodium hydroxide (e.g. concentrations 5N, 1N or other dilutions).

5.4 PBS (phosphate buffered saline), pH 7,3 ± 0,2 [$NaCl/Na_2HPO_4$: 145 mM/10 mM].

5.5 PEG, molecular weight 20 000 g/mol (PolyEthylene Glycol) solution.

Prepare a concentrated PEG solution: weigh 30 g of PEG powder, and add 70 ml of water (5.1).

5.6 Electrode cleaning solution (e.g. ethanol 70 %).

5.7 Immunoenzymatic detection kit dedicated to SEs. Any kit shall comply with the performance criteria in 8.7.

6 Apparatus

Usual microbiological laboratory equipment (in accordance with ISO 7218) and, in particular, the following.

6.1 Blender.

6.2 Balance.

6.3 Homogenization equipment, e.g. rotary homogenizer, blender or peristaltic homogenizer.

It is highly recommended to use a rotary homogenizer, in particular for all types of food difficult to mix in order to obtain a homogeneous sample. If a peristaltic homogenizer is used, only use bags without filter.

6.4 Shaker at room temperature, e.g. orbital shaker, magnetic stirrer, etc.

6.5 pH-meter and electrode, e.g. combination electrode.

6.6 Centrifuge, capable of operating at 3 130*g* minimum; if possible, capable of being refrigerated.

6.7 Dialysis membrane, molecular weight cut off (MWCO) of 6 000 Da to 8 000 Da.

6.8 Closures for dialysis membrane.

6.9 Filtering material, e.g. funnel and cotton-wool, glass-wool, etc.

6.10 Shallow tray.

6.11 Refrigerator (3 °C ± 2 °C or 5 °C ± 3 °C) and **freezer** (≤ −18 °C).

6.12 Laboratory ware in glass or polypropylene to avoid the adsorption of toxins (funnel, beaker, vial, centrifuge tube, etc.).

6.13 Equipment suitable for the detection kit used, see 5.7.

6.14 Water bath (38 °C ± 2 °C).

7 Sampling

Sampling is not part of the method specified in this document.

8 Procedure

8.1 Preparation of test portion

In the case of cheese with rind, take about 10 % of rind and 90 % of core.

As enterotoxins can be heterogeneously distributed in the sample, if possible, mix and homogenize the whole sample or a representative part of it with a blender (6.1). Use 25 g of the homogenized sample as the test portion.

In the case of a suspected staphylococcal food poisoning outbreak (SFPO), the test sample size may be less than 25 g. Perform the analysis as described below and adapt the steps 8.3.1 to 8.5.2 accordingly. The ratio of the weight of the test portion and concentrated extract (8.5.2) should be approximately five [e.g. 25 g test portion for 5,0 g to 5,5 g (maximum 5,8 g for the sticky extracts) of concentrated extract, 12,5 g test portion for 2,5 g to 2,8 g (maximum 2,9 g for the sticky extracts) of concentrated extract].

8.2 Storage of the test sample

It is recommended to store the samples at 3 °C ± 2 °C or 5 °C ± 3 °C (6.11) before analysis.

If analysis is not performed within 24 h, it is possible to freeze the samples. In this case, completely thaw the samples at 3 °C ± 2 °C or 5 °C ± 3 °C before starting the analysis.

To avoid loss of toxins, it is highly recommended not to freeze and thaw the samples repeatedly before analysis.

8.3 Extraction

8.3.1 Add approximately 40 ml of water (5.1) at 38 °C ± 2 °C to the 25 g test portion, except in the case of liquid products. For liquid products, proceed directly as described in 8.3.2. In the case of SFPO, if the test portion is smaller than 25 g, reduce the amount of water (5.1) with the equal ratio.

Homogenize the mixture using a rotary homogenizer or a blender (6.3). This step is particularly important in the case of high fat content products. It is recommended to use a rotary homogenizer for all types of food samples that are difficult to mix in order to obtain a homogeneous sample.

8.3.2 Recover the entire sample and rinse the system (stem of the rotary homogenizer, the stomacher bag or the bowl of the blender) with a minimal volume of water (5.1).

NOTE The greater the volume of liquid used the longer the length of dialysis membrane required.

8.3.3 Allow the toxins to diffuse by shaking the sample (6.4) at room temperature (18 °C to 27 °C) for 30 min to 60 min.

8.3.4 Acidify the mixture with appropriate hydrochloric acid solutions (5.2) in order to obtain a pH between 3,5 and 4,0 measured with a pH meter (6.5).

8.3.5 Centrifuge the entire mixture at 3 130*g* minimum for 15 min under refrigeration temperature (approximately 4 °C) or at room temperature (18 °C to 27 °C) (6.6).

In the case of fatty samples, a centrifugation at refrigeration temperature (approximately 4 °C) is recommended to eliminate the fat particles before the dialysis.

8.3.6 Recover the supernatant in a beaker (6.12). If the supernatant is opaque, repeat centrifugation as described in 8.3.5. After centrifugation pH shall be between 3,0 and 4,5.

If the pH > 4,5, proceed as described in 8.3.4.

If the pH < 3,0, the 3D structure of SEs might be damaged. Take another 25 g test portion and proceed as described in 8.3.1.

8.3.7 Neutralize the mixture with the appropriate sodium hydroxide solutions (5.3) in order to obtain a pH between 7,4 and 7,6.

If pH > 9,0, the 3D structure of SEs might be damaged. Take another 25 g test portion and proceed as described in 8.3.1.

8.3.8 Centrifuge according to 8.3.5.

8.3.9 Recover the entire neutralised aqueous phase for the concentration step.

To recover the maximum amount of toxins, at the end of the acidification and neutralization steps, rinse the electrode and beaker with some drops of water (5.1).

In the case of high fat content samples, the electrode can be cleaned using ethanol 70 % (5.6) to dissolve fat particles after the analysis is complete.

8.3.10 Alternative extraction procedure (optional).

This alternative procedure may only be used in limited circumstances, such as a suspected food poisoning event, and may not be used for milk and milk products. This alternative procedure differs from the described procedure by omitting the dialysis concentration step.

— Take the necessary volume (depending on the kit used) of the neutralized aqueous phase obtained in step 8.3.9 and proceed to the detection step 8.7. Store the remaining neutralized aqueous phase at 3 °C ± 2 °C or 5 °C ± 3 °C.

— If a SEs-negative result is obtained, implement the concentration step (8.4) of the remaining neutralized aqueous phase the same day and repeat the detection using the concentrated extract.

If this procedure is not strictly followed, a new test portion should be analysed.

8.4 Concentration of the extract (mandatory for milk and dairy products)

8.4.1 For each sample, use the PEG solution prepared according to 5.5.

8.4.2 Cut a piece of dialysis membrane (6.7) with sufficient length to contain the entire extract.

8.4.3 Soak the membrane in water (5.1) for rehydration, following the manufacturer's instructions (e.g. at least for 30 min at room temperature).

Before use, rinse the membrane (outside and inner parts) with water (5.1).

8.4.4 Lock one end of the membrane with a closure (6.8).

8.4.5 Fill the prepared membrane with all of the neutralized aqueous phase (8.3.9) using a funnel and a small piece of filtering material (6.9) to filter out suspended particles. Lock the other end of the membrane with a second closure (6.8).

8.4.6 Lay down the filled dialysis membrane in a shallow tray (6.10) filled with the PEG solution (5.5).

8.4.7 Allow the extracts to concentrate, overnight at 3 °C ± 2 °C or 5 °C ± 3 °C (6.11). If the extract is not concentrated enough (i.e. more than 5 ml left in the dialysis membrane), lay it down in the PEG solution for more time (up to 3 days) or add some PEG powder over the membrane.

8.5 Recovery of the concentrated extract

8.5.1 Take the dialysis membrane out of the PEG solution and rinse the outer-parts of the membrane with water (5.1) to remove all traces of PEG solution.

8.5.2 Open one end of the membrane and recover the concentrated extract by rinsing thoroughly the inner-part of the dialysis membrane using

— PBS (5.4) in the case of milk and dairy products, or

— water (5.1) in the case of other matrices.

Rinse thoroughly the inner-parts of the dialysis membrane to obtain a final concentrated extract mass ranging from 5,0 g to 5,5 g (maximum 5,8 g for the sticky extracts).

Carefully transfer the concentrated extract into a glass or polypropylene vial (6.12).

During this critical step, to recover the maximum amount of enterotoxins it is recommended

— to rub the inner-parts of the dialysis membrane (one part against another inner-part) in order to remove and to recover the maximum of SEs, and

— to maximize the quantity of SEs recovered, carry out the recovery of the extract by repeatedly adding small quantities of PBS (5.4) or water (5.1) into the membrane (e.g. add 1 ml or 2 ml), rubbing the membrane as described above and adding the recovered extract into the vial. Repeat these steps until a final mass of 5,0 g to 5,5 (5,8) g per 25 g test portion is obtained.

In the case of a SFPO, the mass of the sample analysed may be lower than 25 g (8.2). The final mass of the concentrated extract (8.5.2) will be adjusted to obtain a final ratio of 1 to 5 between the concentrated extract mass and the test portion mass.

8.6 Storage and steps before detection

If the concentrated extract (8.5.2) will be analysed within 48 h, store it at 3 °C ± 2 °C or 5 °C ± 3 °C (6.11). If the detection cannot be performed within 48 h, store the extract at ≤ −18 °C (6.11) unless otherwise stated by the manufacturer of the detection kit used.

In the case of frozen extract, completely thaw and homogenize it using a vortex before performing the detection step.

If foaming appears, make sure to pipet in the liquid phase.

8.7 Detection

Select a detection kit that fulfils the performance criteria (sensitivity, specificity, LOD_{50}) for the entire procedure, defined in this document (see 8.8).

Carefully follow the manufacturer's instructions for the detection step of the kit used.

8.8 Performance criteria

Performance criteria including specificity (SP, 3.2), sensitivity (SE, 3.3), limit of detection 50 % (LOD_{50}, 3.4) have been defined for the entire procedure, including extraction and detection. The calculation for LOD_{50} was performed using a dedicated programme available from the ISO website.

The performance criteria that the commercial kits shall achieve are defined as follows:

— SP and SE should be higher than 90 %.

— LOD_{50} should be less than 0,06 ng SEs/g. This value is based on the estimated BMD for SEA of 6,1 ng and the assumed ingestion of 100 g of food.

As consolidated data were only available for SEA, the staphylococcal enterotoxin most frequently involved in SFPO, it was decided to use this value for the other toxin types SEB to SEE.[4]

Values obtained by different detection kits and food matrices with and without dialysis are presented in Clause 12. Laboratories shall refer to the data obtained to perform the selection of the detection kit which fulfils the criteria mentioned above.

The data are summarized in Annexes A and B for the interlaboratory studies organized in 2013 and 2014, respectively. The values derived from the interlaboratory studies may not be applicable to food types other than those given in Annexes A and B.

9 Quality control

It is recommended to check the entire procedure, with reference materials. An example of a suitable reference material is given in Reference [5].

10 Expression of results

Express the results of the screening method as

— staphylococcal enterotoxins SEA to SEE detected in *x* g of the test portion, or

— staphylococcal enterotoxins SEA to SEE not detected in *x* g of the test portion.

11 Confirmation

For a positive result obtained with or without dialysis concentration, it is recommended that a related sample is analysed for confirmatory purposes, using a different method than the one described in this document**,** as it is well known that interferences may occur (see Annex C).

12 Performance characteristics of the method

The performance characteristics of the method were determined in interlaboratory studies to evaluate the specificity, sensitivity and the LOD_{50} of the method.

Data obtained for specificity, sensitivity and LOD_{50} are presented on Tables 1, 2 and 3, respectively.

Table 1 — Specificity (%) values obtained by the three kits tested on the five food categories

Matrices	Detection kits	+DC	-DC
Ready to eat food (RTE) (SEA)	Vidas SET2[a]	100	100
	Ridascreen SET Total[b]	100	100
	Tecra Staph VIA[c]	90	100
Fish product (SEC)	Vidas SET2	100	100
	Ridascreen SET Total	100	100
	Tecra Staph VIA	100	80
Dessert (SEE)	Vidas SET2	100	100
	Ridascreen SET Total	100	100
	Tecra Staph VIA	100	100
Cheese (SED)	Vidas SET2	98	Not performed
	Ridascreen SET Total	100	
	Tecra Staph VIA	100	
Meat product (SEA)	Vidas SET2	100	100
	Ridascreen SET Total	100	100
	Tecra Staph VIA	93	100

Key

\+ DC: With dialysis concentration.

-DC: Without dialysis concentration.

[a] Vidas SET2 is a product available commercially and supplied by bioMérieux SA, Marcy l'Etoile, France. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

[b] Ridascreen SET Total is a product available commercially and supplied by R-biopharm AG, Darmstadt, Germany. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

[c] Tecra Staph VIA was a product available commercially and supplied by 3M, Saint Paul, MN, United States. It has been withdrawn from the market. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

Table 2 — Sensitivity (%) values obtained by the three kits tested on the five food categories

		RTE (SEA)		Fish (SEC)		Dessert (SEE)		Cheese (SED)		Meat (SEA)	
kit	**level**	+DC	-DC	+DC	-DC	+DC	-DC	+DC	-DC	+DC	-DC
Vidas SET2	**L1**	100	87	100	3	100	1	100	—	100	99
	L2	100	100	100	68	100	86	100	—	100	100
Ridascreen SET total	**L1**	98	10	98	10	94	0	87	—	100	37
	L2	100	52	100	44	100	26	98	—	100	100
Tecra Staph VIA	**L1**	33	10	97	23	20	0	83	—	95	10
	L2	57	17	100	30	37	10	97	—	100	100
	L3	100	30	—	—	50	10	—	—	—	—

Key

\+ DC: With dialysis concentration.

-DC: Without dialysis concentration.

—: Not performed.

NOTE L1, L2, L3 correspond to different levels of contamination for evaluation of performance characteristics depending on detection kits and type of samples. See tables in Annex A for details.

Table 3 — LOD_{50} (ng/g) values obtained by the three kits tested on the five food categories

LOD_{50} ng/g	RTE food (SEA)		Fish (SEC)		Pastry (SEE)		Cheese (SED)	Meat (SEA)	
	+DC	-DC	+DC	-DC	+DC	-DC	+DC	+DC	-DC
Vidas SET 2	0,007	0,019	0,017	0,552	0,017	0,375	—	0,007	0,015
Ridascreen SET Total	0,010	0,184	0,027	0,815	0,036	1,444	0,076	0,010	0,120
Tecra Staph VIA	0,082	0,406	0,031	0,35	0,408	2,673	0,092	0,082	0,211

Key

\+ DC: With dialysis concentration.

-DC: Without dialysis concentration.

—: Not calculated with naturally contaminated samples, level above LOD_{50}.

13 Test report

The following wording may be used as a model, with extra entries added on a case-by-case basis.

The test report shall contain at least the following information:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, with reference to this document, i.e. ISO 19020;

d) all operating details not specified in this document, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

e) the test result(s) obtained.

Annex A
(informative)

Results of interlaboratory studies: 2013

An international interlaboratory study involving 21 collaborators in 17 countries was carried out. The following food types were involved in the study: cheese corresponding to the milk and milk product category and poultry corresponding to the meat category. The food samples were each tested at two different levels of contamination (L1 and L2), plus a negative control (L0). The study was organized in 2013 by the European Union Reference Laboratory for CPS.

Following a preliminary study organized from February to May 2013, three detection kits (Ridascreen SET Total; Vidas SET2; Tecra Staph Enterotoxin VIA)[1)] from the seven tested were selected based on their performance on five types of food matrices. These three selected kits were evaluated during the 2013 interlaboratory study on the two food matrices mentioned above.

The following batch numbers were used by participating laboratories:

— Ridascreen SET Total: 12362

— Vidas SET2: 140412-0 and 140614-0

— Tecra Staph VIA: 12212012 and 16212084

The values of the performance characteristics derived from this interlaboratory study are shown per type of sample in Tables A.1 to A.9. Data obtained by some collaborators have been excluded from the calculations on the basis of clearly identified technical reasons (e.g. deviations to the protocol).

Results obtained on milk and milk products (Tables A.1 to A.3) refer only to the dialysis concentration step as this step is mandatory in EU Regulation EC 2073/2005 modified by EC 1441/2007 for these types of food matrices.

1) Ridascreen SET Total is a product available commercially and supplied by R-biopharm AG, Darmstadt, Germany. Vidas SET2 is a product available commercially and supplied by bioMérieux SA, Marcy l'Etoile, France. Tecra VIA was a product available commercially and supplied by 3M, Saint Paul, MN, United States; it has been withdrawn from the market. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

Table A.1 — Results of data analysis obtained with Ridascreen SET Total with dialysis concentration on cheese samples

Parameter	L0 Blank	L1 low level SED at 0,2 ng/g	L2 high level SED at 0,5 ng/g
Number of participating collaborators	17	17	17
Number of collaborators retained after evaluation of the data	16	16	16
Number of samples	64	128	64
Number of samples retained after evaluation of the data	62	123	64
Sample size (g)	25	25	25
Sensitivity (%)	—	100	100
Specificity (%)	100	—	—
LOD_{50} (ng/g)	Calculation not possible as 100 % accuracy for L1 and L2		
—: Not relevant.			

Table A.2 — Results of data analysis obtained with Tecra Staph VIA with dialysis concentration on cheese samples

Parameter	L0 Blank	L1 low level SED at 0,2 ng/g	L2 high level SED at 0,5 ng/g
Number of participating collaborators	17	17	17
Number of collaborators retained after evaluation of the data	16	16	16
Number of samples	64	128	64
Number of samples retained after evaluation of the data	61	120	62
Sample size (g)	25	25	25
Sensitivity (%)	—	95	100
Specificity (%)	93	—	—
LOD_{50} (ng/g)	LOD_{50}: 0,022		
—: Not relevant.			

Table A.3 — Results of data analysis obtained with Vidas SET2 with dialysis concentration on cheese samples

Parameter	L0 Blank	L1 low level SED at 0,2 ng/g	L2 high level SED at 0,5 ng/g
Number of participating collaborators	16	16	16
Number of collaborators retained after evaluation of the data	16	16	16
Number of samples	64	128	64
Number of samples retained after evaluation of the data	63	123	64
Sample size (g)	25	25	25
Sensitivity (%)	—	100	100
Specificity (%)	100	—	—
LOD_{50} (ng/g)	Calculation not possible as 100 % accuracy for L1 and L2		
—: Not relevant.			

Table A.4 — Results of data analysis obtained with Ridascreen SET Total with dialysis concentration on meat samples

Parameter	L0 Blank	L1 low level SEA at 0,1 ng/g	L2 high level SEA at 0,5 ng/g
Number of participating collaborators	17	17	17
Number of collaborators retained after evaluation of the data	16	16	16
Number of samples	64	128	64
Number of samples retained after evaluation of the data	62	123	64
Sample size (g)	25	25	25
Sensitivity (%)	—	100	100
Specificity (%)	100	—	—
LOD_{50} (ng/g)	Calculation not possible as 100 % accuracy for L1 and L2		
—: Not relevant.			

Table A.5 — Results of data analysis obtained with Ridascreen SET Total without dialysis concentration on meat samples

Parameter	L0 Blank	L1 low level SEA at 0,1 ng/g	L2 high level SEA at 0,5 ng/g
Number of participating collaborators	16	16	16
Number of collaborators retained after evaluation of the data	15	15	15
Number of samples	60	120	60
Number of samples retained after evaluation of the data	56	111	60
Sample size (g)	25	25	25
Sensitivity (%)	—	37	100
Specificity (%)	100	—	—
LOD_{50} (ng/g)	LOD_{50}: 0,120		
—: Not relevant.			

Table A.6 — Results of data analysis obtained with Tecra Staph VIA with dialysis concentration on meat samples

Parameter	L0 Blank	L1 lox level SEA at 0,1 ng/g	L2 high level SEA at 0, 5 ng/g
Number of participating collaborators	17	17	17
Number of collaborators retained after evaluation of the data	16	16	16
Number of samples	64	128	64
Number of samples retained after evaluation of the data	61	120	62
Sample size (g)	25	25	25
Sensitivity (%)	—	95	100
Specificity (%)	93	—	—
LOD_{50} (ng/g)	LOD_{50}: 0,022		
—: Not relevant.			

Table A.7 — Results of data analysis obtained with Tecra Staph VIA without dialysis concentration on meat samples

Parameter	L0 Blank	L1 low level SEA at 0,1 ng/g	L2 high level SEA at 0,5 ng/g
Number of participating collaborators	17	17	17
Number of collaborators retained after evaluation of the data	16	16	16
Number of samples	64	128	64
Number of samples retained after evaluation of the data	60	117	62
Sample size (g)	25	25	25
Sensitivity (%)	—	10	100
Specificity (%)	100	—	—
LOD_{50} (ng/g)	LOD_{50}: 0,211		
—: Not relevant.			

Table A.8 — Results of data analysis obtained with Vidas SET2 with dialysis concentration on meat samples

Parameter	L0 Blank	L1 low level SEA at 0,1 ng/g	L2 high level SEA at 0,5 ng/g
Number of participating collaborators	16	16	16
Number of collaborators retained after evaluation of the data	16	16	16
Number of samples	64	128	64
Number of samples retained after evaluation of the data	63	123	64
Sample size (g)	25	25	25
Sensitivity (%)	—	100	100
Specificity (%)	100	—	—
LOD_{50} (ng/g)	Calculation not possible as 100 % accuracy for L1 and L2		
—: Not relevant.			

Table A.9 — Results of data analysis obtained with Vidas SET2 without dialysis concentration on meat samples

Parameter	L0 Blank	L1 low level SEA at 0,1 ng/g	L2 high level SEA at 0,5 ng/g
Number of participating collaborators	16	16	16
Number of collaborators retained after evaluation of the data	16	16	16
Number of samples	64	128	64
Number of samples retained after evaluation of the data	63	123	64
Sample size (g)	25	25	25
Sensitivity (%)	—	99	100
Specificity (%)	100	—	—
LOD_{50} (ng/g)	LOD_{50}: 0,015		
—: Not relevant.			

Annex B
(informative)

Results of interlaboratory studies: 2014

An international interlaboratory study involving 33 collaborators in 20 countries was carried out. The following food types were involved in the study: mackerel corresponding to the fish product category, quiche Lorraine corresponding to the ready to eat (RTE) food category and dessert cream corresponding to the dessert category. The food samples were each tested at two different levels of contamination (L1 and L2), plus a negative control (L0). The study was organized in 2014 by the European Union Reference Laboratory for CPS.

The aim of this second part of the interlaboratory study was to complete data on the three remaining food categories to be tested for the three detection kits (Ridascreen SET Total, Tecra Staph VIA and Vidas SET2). [2)]

The following batch numbers were used by participating laboratories:

— Ridascreen SET Total: 14393

— Tecra Staph VIA: 16213010

— Vidas SET2: 140902-0

The values of the performance characteristics derived from this interlaboratory study are shown per type of sample in Tables B.1 to B.9.

Tables B.1 to B.3 correspond to the fish product category with and without dialysis concentration.

Tables B.4 to B.6 correspond to the RTE category with and without dialysis concentration.

Tables B.7 to B.9 correspond to the dessert category with and without dialysis concentration.

Data obtained by some collaborators have been excluded from the calculations on the basis of clearly identified technical reasons (e.g. deviations to the protocol).

2) Ridascreen SET Total is a product available commercially and supplied by R-biopharm AG, Darmstadt, Germany. Vidas SET2 is a product available commercially and supplied by bioMérieux SA, Marcy l'Etoile, France. Tecra VIA was a product available commercially and supplied by 3M, Saint Paul, MN, United States; it has been withdrawn from the market. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

Table B.1 — Results of data analysis obtained with Ridascreen SET Total on fish samples with and without dialysis concentration

Parameter	Analysis with DC			Analysis without DC		
	L0 Blank	L1 SEC 0,15 ng/g	L2 SEC 0,25 ng/g	L0 Blank	L1 SEC 0,15 ng/g	L2 SEC 0,25 ng/g
Number of participating laboratories	17	17	17	17	17	17
Number of laboratories retained after evaluation of the data	17	17	17	17	17	17
Number of samples	17	51	17	17	51	17
Number of samples retained after evaluation of the data	17	51	17	16	50	16
Sample size (g)	25	25	25	25	25	25
Sensitivity (%)	—	98	100	—	10	44
Specificity (%)	100	—	—	100	—	—
LOD_{50} (ng/g)	**LOD_{50}: 0,027**			**LOD_{50}: 0,815**		
—: Not relevant.						

Table B.2 — Results of data analysis obtained with Tecra Staph VIA on fish samples with and without dialysis concentration

Parameter	Analysis with DC			Analysis without DC		
	L0 Blank	L1 SEC 0,15 ng/g	L2 SEC 0,25 ng/g	L0 Blank	L1 SEC 0,15 ng/g	L2 SEC 0,25 ng/g
Number of participating laboratories	11	11	11	11	11	11
Number of laboratories retained after evaluation of the data	10	10	10	10	10	10
Number of samples	11	33	11	11	33	11
Number of samples retained after evaluation of the data	10	30	10	10	30	10
Sample size (g)	25	25	25	25	25	25
Sensitivity (%)	—	97	100	—	23	30
Specificity (%)	100	—	—	80	—	—
LOD_{50} (ng/g)	LOD_{50}: 0,031			LOD_{50}: 0,350		
—: Not relevant.						

Table B.3 — Results of data analysis obtained with Vidas SET2 on fish samples with and without dialysis concentration

Parameter	Analysis with DC			Analysis without DC		
	L0 Blank	L1 SEC 0,15 ng/g	L2 SEC 0,25 ng/g	L0 Blank	L1 SEC 0,15 ng/g	L2 SEC 0,25 ng/g
Number of participating laboratories	25	25	25	25	25	25
Number of laboratories retained after evaluation of the data	25	25	25	25	25	25
Number of samples	25	75	25	25	75	25
Number of samples retained after evaluation of the data	25	75	25	25	75	25
Sample size (g)	25	25	25	25	25	25
Sensitivity (%)	—	100	100	—	3	68
Specificity (%)	100	—	—	100	—	—
LOD_{50} (ng/g)	LOD_{50}: 0,017			LOD_{50}: 0,552		
—: Not relevant.						

Table B.4 — Results of data analysis obtained with Ridascreen SET Total on RTE samples with and without dialysis concentration

Parameter	Analysis with DC			Analysis without DC		
	L0 Blank	L1 SEA 0,055 ng/g	L2 SEA 0,11 ng/g	L0 Blank	L1 SEA 0,055 ng/g	L2 SEA 0,11 ng/g
Number of participating laboratories	17	17	17	17	17	17
Number of laboratories retained after evaluation of the data	17	17	17	17	17	17
Number of samples	17	51	23	17	51	23
Number of samples retained after evaluation of the data	17	51	23	17	51	23
Sample size (g)	25	25	25	25	25	25
Sensitivity (%)	—	98	100	—	10	52
Specificity (%)	100	—	—	100	—	—
LOD_{50} (ng/g)	LOD_{50}: 0,010			LOD_{50}: 0,184		
—: Not relevant.						

Table B.5 — Results of data analysis obtained with Tecra Staph VIA on RTE samples with and without dialysis concentration

Parameter	Analysis with DC				Analysis without DC			
	L0 Blank	L1 SEA 0,055 ng/g	L2 SEA 0,11 ng/g	L3 SEA 0,22 ng/g	L0 Blank	L1 SEA 0,055 ng/g	L2 SEA 0,11 ng/g	L3 SEA 0,22 ng/g
Number of participating laboratories	11	11	11	11	11	11	11	11
Number of laboratories retained after evaluation of the data	10	10	10	10	10	10	10	10
Number of samples	11	33	33	11	11	33	33	11
Number of samples retained after evaluation of the data	10	30	30	10	10	30	30	10
Sample size (g)	25	25	25	25	25	25	25	25
Sensitivity (%)	—	33	57	100	—	10	17	30
Specificity (%)	90	—	—	—	100	—	—	—
LOD_{50} (ng/g)	LOD_{50}: 0,082				LOD_{50}: 0,406			
—: Not relevant.								

Table B.6 — Results of data analysis obtained with Vidas SET2 on RTE samples with and without dialysis concentration

Parameter	Analysis with DC			Analysis without DC		
	L0 Blank	L1 SEA 0,055 ng/g	L2 SEA 0,11 ng/g	L0 Blank	L1 SEA 0,055 ng/g	L2 SEA 0,11 ng/g
Number of participating laboratories	25	25	25	25	25	25
Number of laboratories retained after evaluation of the data	25	25	25	25	25	25
Number of samples	25	75	29	25	75	29
Number of samples retained after evaluation of the data	25	75	29	25	75	29
Sample size (g)	25	25	25	25	25	25
Sensitivity (%)	—	100	100	—	87	100
Specificity (%)	100	—	—	100	—	—
LOD_{50} (ng/g)	LOD_{50}: 0,007			LOD_{50}: 0,019		
—: Not relevant.						

Table B.7 — Results of data analysis obtained with Ridascreen SET Total on dessert samples with and without dialysis concentration

Parameter	Analysis with DC			Analysis without DC		
	L0 Blank	L1 SEE 0,15 ng/g	L2 SEE 0,25 ng/g	L0 Blank	L1 SEE 0,15 ng/g	L2 SEE 0,25 ng/g
Number of participating laboratories	17	17	17	17	17	17
Number of laboratories retained after evaluation of the data	17	17	17	17	17	17
Number of samples	17	51	23	17	51	23
Number of samples retained after evaluation of the data	17	50	23	17	50	23
Sample size (g)	25	25	25	25	25	25
Sensitivity (%)	—	94	100	—	0	26
Specificity (%)	100	—	—	100	—	—
LOD_{50} (ng/g)	LOD_{50}: 0,036			LOD_{50}: 1,444		
—: Not relevant.						

Table B.8 — Results of data analysis obtained with Tecra Staph VIA on dessert samples with and without dialysis concentration

Parameter	Analysis with DC				Analysis without DC			
	L0 Blank	L1 SEE 0,15 ng/g	L2 SEE 0,25 ng/g	L3 SEE 0,40 ng/g	L0 Blank	L1 SEE 0,15 ng/g	L2 SEE 0,25 ng/g	L3 SEE 0,40 ng/g
Number of participating laboratories	11	11	11	11	11	11	11	11
Number of laboratories retained after evaluation of the data	10	10	10	10	10	10	10	10
Number of samples	11	33	33	11	11	33	33	11
Number of samples retained after evaluation of the data	10	30	30	10	10	30	30	10
Sample size (g)	25	25	25	25	25	25	25	25
Sensitivity (%)	—	20	37	50	—	0	10	10
Specificity (%)	100	—	—	—	100	—	—	—
LOD_{50} (ng/g)	LOD_{50}: 0,408				LOD_{50}: 2,673			
—: Not relevant.								

Table B.9 — Results of data analysis obtained with Vidas SET2 on dessert samples with and without dialysis concentration

Parameter	Analysis with DC			Analysis without DC		
	L0 Blank	L1 SEE 0,15 ng/g	L2 SEE 0,25 ng/g	L0 Blank	L1 SEE 0,15 ng/g	L2 SEE 0,25 ng/g
Number of participating laboratories	25	25	25	25	25	25
Number of laboratories retained after evaluation of the data	25	25	25	25	25	25
Number of samples	25	75	29	25	75	29
Number of samples retained after evaluation of the data	25	75	29	25	75	29
Sample size (g)	25	25	25	25	25	25
Sensitivity (%)	—	100	100	—	1	86
Specificity (%)	100	—	—	100	—	—
LOD_{50} (ng/g)	LOD_{50}: 0,017			LOD_{50}: 0,375		
—: Not relevant.						

Annex C
(informative)

Note on interferences

C.1 It is well known that the immunological detection of staphylococcal enterotoxins in food matrices has several drawbacks. Non-specific SEs reaction with some of the commercially available kits were previously reported with various food types or with food contaminated by microorganisms other than *Staphylococcus* spp. Some interferences can be attributed to endogenous enzymes such as lactoperoxidase (see C.3) or alkaline phosphatase coming from raw milk (see C.2).

C.2 The alkaline phosphatase is usually present in cheeses made from raw milk.

In the case of positive result by a detection kit using alkaline phosphatase as enzyme (e.g. Vidas SET2) and if interferences are suspected:

a) place 600 µl of treated concentrated extract in a tube;

b) perform a heat-treatment at 80 °C during two minutes (to destroy alkaline phosphatase),

c) after cooling, perform a new detection using a Vidas SET2 detection kit.

As this heat-treatment can lead to a loss of serological activity of the enterotoxins present in the concentrated extract, it is not performed before the detection step due to possible false-negative results.

However, in the case of a positive result obtained by the Vidas SET2 detection kit and if interferences are suspected, such a procedure can be applied.

In the case of suspected interferences with Vidas SET2 kit, perform if possible another detection kit using another enzyme as detection (e.g. lactoperoxidase used in the Ridascreen SET Total kit).

C.3 The lactoperoxidase is resistant to pH 3 and its molecular weight could explain the fact that it is not destroyed or inactivated during the extraction step.

In the case of a positive result obtained by a detection kit using lactoperoxidase as enzyme (e.g. Ridascreen SET Total) and if interferences due to endogenous lactoperoxidase are suspected, the following test can be performed:

a) place 100 µl of treated concentrated extract in a tube and add 50 µl of substrate and 50 µl of chromogenic solutions from the Ridascreen SET Total detection kit;

b) if a blue-green colour appears, endogenous lactoperoxidase is present in the concentrated extract and can explain a false-positive result obtained with the screening method.

If interferences with Ridascreen SET Total detection kit are suspected, perform, if possible, the detection with a kit using another enzyme.

Bibliography

[1] Hennekinne J.-A., De Buyser M.-L., Dragacci S. *Staphylococcus aureus* and its food-poisoning toxins: characterization and outbreak investigation. *FEMS Microbiol. Rev*. 2012, **36** pp. 815–836

[2] COMMISSION REGULATION (EC) . No. 1441/2007 of 5 December 2007 amending Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs. *Off J. Eur. Union.* 2007, **L322** pp. 12–29

[3] Guillier L., Bergis H., Guillier F., Noel V., Auvray F., Hennekinne J.A. Dose-response modelling of staphylococcal enterotoxins using outbreak data. *Procedia Food Sci.* 2016, **7** pp. 129–132

[4] Davis J.-A., Gift J.-S., Zhao Q.-J. Introduction to benchmark dose methods and US EPA's benchmark dose software (BMDS) version 2.1. 1. *Toxicol. Appl. Pharmacol*. 2011, **254** pp. 181–191

[5] Zeleny R., Nia Y., Schimmel H., Mutel I., Hennekinne J.-A., Emteborg H, Charoud-Got J, Auvray F Certified reference materials for testing of the presence/absence of *Staphylococcus aureus* enterotoxin A (SEA) in cheese. *Anal. Biochem.* 2016, **408** pp. 5457–5465

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