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Microbiology of the food chain — Horizontal method for the immunoenzymatic detection of staphylococcal enterotoxins in foodstuffs

Microbiologie de la chaîne alimentaire — Méthode horizontale de détection des entérotoxines staphylococciques par test immuno-enzymatique dans les aliments

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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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.

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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

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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.

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