# PERFORMANCE OF AN IMMUNOASSAY-BASED METHOD FOR MONITORING THE STAPHYLOCOCCAL ENTEROTOXIN PRODUCTION IN FOOD AND CULTURE SUPERNATANT

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## ABSTRACT

Staphylococcal enterotoxins (SEs) are among the leading causes of bacterial food poisoning in France, economically important, involving medical expenses and loss of productivity. They are mainly produced by *Staphylococcus aureus* species, they are resistant to heat treatment, to proteolytic enzymes, and are quite stable over a wide range of pH.

We developed a sandwich-type ELISA, called Transia Plate *Staphylococcal Enterotoxins*, allowing the detection of the 7 main enterotoxins (SEA, SEB, SEC1, SEC2, SEC3, SED and SEE) in food, within 2 hours after sample preparation. For these 7 enterotoxins diluted in buffer solution, the detection limit of the immunoassay was at least equal to or lower than 0.1 ng SE/ml.

The most frequently incriminated food products in staphylococcal food poisoning (eggs, mayonnaise, cake, fresh meat, canned fish, paella and ravioli, as well as dairy products) were spiked with different levels of SEA (0, 0.25, 0.5, 1 and 2 ng/g of product). The detection limit varied according to the matrix and was respectively found at 0.25 ng of SEA/g of sample for milk, mayonnaise and paella, at 0.5 ng SEA/g for cheese, cream cake, ravioli and canned fish, and around 1 ng SEA/g for fresh meat. These results confirmed the immunoassay could detect the presence of enterotoxin in various complex food matrices.

We also studied the production of 2 enterotoxins (SEA and SED) by 2 different *Staphylococcus aureus* strains inoculated in a brain heart culture containing food samples (milk and cheese). The enterotoxin production started to be detected after 3 hours of growth, when the cell concentration was around $10^4$ CFU/ml for both strains: both SEs were excreted during the exponential growth.

The Transia Plate *Staphylococcal Enterotoxin* assay could be applied for routine screening of food samples and also for monitoring the conditions of enterotoxin production in food.

# *Performance of an Immunoassay-based Method for monitoring the Staphylococcal Enterotoxin Production in Food and Culture Supernatant*

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**ABSTRACT** :

Staphylococcal enterotoxins (SEs) are among the leading causes of bacterial food poisoning in France, economically important, involving medical expenses and loss of productivity. They are mainly produced by *Staphylococcus aureus* species, they are resistant to heat treatment, to proteolytic enzymes, and are quite stable over a wide range of pH. We developed a sandwich-type ELISA, called Transia Plate *Staphylococcal Enterotoxins*, allowing the detection of the 7 main enterotoxins (SEA, SEB, SEC1, SEC2, SEC3, SED and SEE) in food, within 2 hours after sample preparation. For these 7 enterotoxins diluted in buffer solution, the detection limit of the immunoassay was at least equal to or lower than 0.1 ng SE/ml.

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**INTRODUCTION** :

Staphylococcal Enterotoxins (SEs) have been reported as causative agents of food poisoning outbreaks worldwide. These bacterial toxins are mostly produced by *Staphylococcus aureus* and are present in various foods such as dairy products, raw and processed meat and also canned food. Currently, seven serological types of SEs (A, B, C1, C2, C3, D and E) have been clearly identified regarding their antigenic characteristics, their genomic sequences and their prevalence in human outbreaks. Their detection in food samples is usually carried out with immunoassays, after a sample extraction step. In the present work, we evaluated the performances of the Transia Plate *Staphylococcal Enterotoxins* assay for the detection of the SEs in various food matrices. We, then, studied the production of the SEs by *Staphylococcus aureus* in brain heart infusion broth (BHI) or saline solution with and without presence of food matrix.

**MATERIALS AND METHODS** :

**Transia Plate *Staphylococcal Enterotoxins* (TP SE) :**

It is a sandwich ELISA allowing the detection of the 7 main SEs by using a mixture of monoclonal and polyclonal antibodies. Briefly, the assay was performed in 3 steps (sample incubation, conjugate incubation and colorimetric revelation), for an overall assay time of 2 hours, including sample dispensing and washing steps. The optical densities (ODs) were measured at 450 nm and the assay cut off was calculated as the mean of the negative control ODs plus 0.20. In order to determine the detection limit of the assay for 5 SEs (A, B, C3, D and E), we tested different SE concentrations (from 0.01 ng/ml to 1 ng/ml) prepared in PBS.

**Culture of *Staphylococcus aureus* in BHI :**

Two strains of *Staphylococcus aureus* (574 and 819), producing respectively SEA and SED, were grown in BHI, at 37°C during 24 hours. Both strains were inoculated at a cell level of $10^4$-$10^5$ CFU/ml. From 0 to 9 hours, sample of 1 ml was taken up for the SE measurement by ELISA and for the *Staphylococcus aureus* enumeration on Baird Parker agar.

**Culture of *Staphylococcus aureus* in saline solution containing food :**

Three different food matrices were studied in solution saline : pasteurized milk and two raw milk cheeses ("Tomme de Savoie" and "Brie"). Twenty five grams of food were mixed to 25 ml of saline solution and cultured at 37°C during 24 hours. Samples were analyzed after 0, 8 and 24 hour incubation for the *Staphylococcus aureus* enumeration and SEs detection by ELISA.

**Spiked food samples :**

SEA was used at different concentrations (0, 0.25, 0.5, 1 and 2 ng/ml) for spiking 3 categories of food matrices : raw meat, dairy products and other products. Various sample extraction procedures were applied according to the food type.

**RESULTS AND DISCUSSION :**

**Detection of the enterotoxins :**

In buffer solution, the 5 enterotoxins (SEA, SEB, SEC3, SED and SEE) were detected by the Transia Plate *Staphylococcal Enterotoxins* immunoassay in less than 2 hours. The detection limit for each enterotoxin was found equal to 0.1 ng/ml for SEE, and lower than 0.05 ng/ml for SEA, SEB, SEC3 and SED.

**Study of Spiked Food Samples (table 1) :**

The Transia Plate *Staplylococcal Enterotoxins* method allowed the detection of the SEA in all the complex food matrices. The detection limit was found between 0.25 ng/ml and 1 ng/ml depending on the food matrix. The variation of the detection limits could be explained by differences in SE extraction yields obtained for the various types of food. The interaction of SE with various food compounds and the phenomena of precipitation and denaturation during the sample preparation step certainly contributed to these differences of extraction yields.

| Food matrix | Sensitivity |
|---|---|
| **Paella** | **0.25 ng/ml** |
| **Mayonnaise** | **0.25 ng/ml** |
| **Fresh eggs** | **0.5 ng/ml** |
| **Raviolis** | **0.5 ng/ml** |
| **Canned tuna** | **0.5 ng/ml** |
| **Confectionery product** | **0.5 ng/ml** |

| Food matrix | Sensitivity |
|---|---|
| **Brie** | **0.5 ng/ml** |
| **Tomme de Savoie** | **0.5 ng/ml** |
| **Pasteurized Milk** | **0.25 ng/ml** |

| Food matrix | Sensitivity |
|---|---|
| **Chicken** | **1 ng/ml** |
| **Veal** | **1 ng/ml** |
| **Pork** | **1 ng/ml** |
| **Beef** | **1 ng/ml** |

**Table 1.** SEA detection in spiked foods.

**Culture of *Staphylococcus aureus* in BHI (figure 1) :**

For both strains, the SEs started being significantly detected after 2 to 3 hours of incubation and that production increased regularly, following the strain growth. The SE production by *Staphylococcus aureus* started during exponential growth and was pursued up to the plateau level. After 9h incubation, both strains were close to their maximum cell concentration (respectively 1.5 $10^8$ CFU/ml at 9h and 1.9 $10^8$ CFU/ml at 24h for the strain 574, and 4.5 $10^8$ CFU/ml at 9h and 6.6 $10^8$ CFU/ml at 24h for the strain 819), but only the production of SEA with strain 574 still increased very significantly between 9 and 24 hours, from 26 to 73 ng/ml.

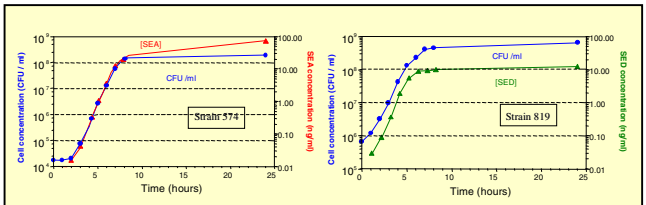


**Figure 1**. Production of SEA or SED during the growth of *S. aureus* (574 or 819) in BHI at 37°C.

**Culture of *Staphylococcus aureus* in saline solution containing food (figure 2) :**

In the presence of pasteurized milk, both strains reached a cell concentration higher than $10^9$ CFU/ml after 24 hours, relatively close to the results obtained previously in BHI. The SE production, reported here in OD, followed the cell growth at 8 and 24 hours but did not reach the concentrations obtained previously in BHI. After 24 hour incubation, the SE concentrations were evaluated respectively at 12.5 ng SEA/ml and 4.25 ng SED/ml : in BHI for the same incubation time, the SE concentrations were higher (respectively 73 ng SEA/ml and 12.3 ng SED/ml) despite of lower cell concentrations.

In the presence of cheese, both strains did grow more slowly than in milk solution, and reached only cell concentration of $10^6$-$10^7$ CFU/ml after 24 hours. These lower concentrations could certainly be explained by inhibition from compounds present in the cheeses and by competition from their regular background flora. Furthermore, the SEA production was not detected at 8 hours but only at 24 hours, showing also a delay in SE production. SED could not be detected at all, despite of a significant growth of *Staphylococcus aureus*. It showed the SE production was also affected by environmental factors.

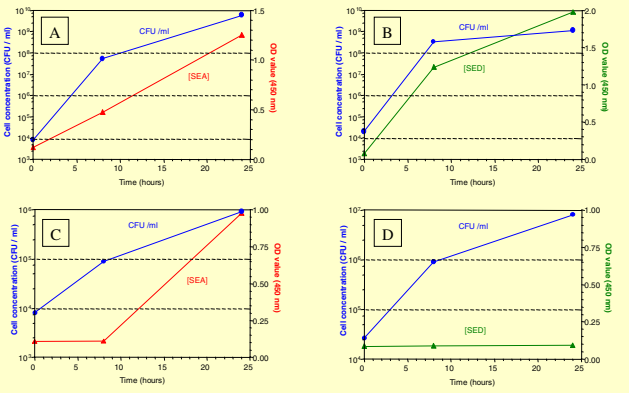


**Figure 2.** Growth of *S. aureus* (819 or 574) and production of SEA (strain 574) or SED (strain 819) in saline solution at 37°C, in presence of pasteurized milk (2-A and 2-B), "Tomme" cheese (2-C) or "Brie" cheese (2-D).

**CONCLUSION :**

The Transia Plate *Staphylococcal Enterotoxins* method detected low levels of staphylococcal enterotoxins in various food matrices, and represented an interesting tool for routine controls of SE contamination in foodstuff. This study confirmed also that the production of SEs was dependent on various environmental factors such as background flora and chemical compounds. The presence of enterotoxigenic *S. aureus* strains in food should not always result in the enterotoxin production.

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