Journal of Food Protection, Vol. 68, No. 6, 2005, Pages 1264–1270

Staphylococcal Enterotoxin and Its Rapid Identification in Foods by Enzyme-Linked Immunosorbent Assay–Based Methodology

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MS 04-703: Received 10 May 2004/Accepted 19 September 2004

ABSTRACT

The problem of *Staphylococcus aureus* and other species as contaminants in the food supply remains significant on a global level. Time and temperature abuse of a food product contaminated with enterotoxigenic staphylococci can result in formation of enterotoxin, which can produce foodborne illness when the product is ingested. Between 100 and 200 ng of enterotoxin can cause symptoms consistent with staphylococcal intoxication. Although humans are the primary reservoirs of contamination, animals, air, dust, and food contact surfaces can serve as vehicles in the transfer of this pathogen to the food supply. Foods may become contaminated during production or processing and in homes or food establishments, where the organism can proliferate to high concentrations and subsequently produce enterotoxin. The staphylococcal enterotoxins are highly heat stable and can remain biologically active after exposure to retort temperatures. Prior to the development of serological methods for the identification of enterotoxin, monkeys (gastric intubation) and later kittens (intravenous injection) were used in assays for toxin detection. When enterotoxins were identified as mature proteins that were antigenic, serological assays were developed for use in the laboratory analysis of foods suspected of containing preformed enterotoxin. More recently developed methods are tracer-labeled immunoassays. Of these methods, the enzyme-linked immunosorbent assays are highly specific, highly sensitive, and rapid for the detection of enterotoxin in foods.

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The recognition of staphylococci as pathogens that cause human infections dates back to biblical times. The first association of *Staphylococcus* with foodborne illness occurred as early as 1884. This organism was found in cheese incriminated in a large outbreak of food poisoning in the United States. Other reported outbreaks attributed to the consumption of foods contaminated with staphylococci occurred in France in 1894, in Michigan in 1907, and the Philippines in 1914. In 1930, Dack et al. *(26)* at the University of Chicago were able to demonstrate that the cause of food poisoning that occurred after the consumption of a contaminated sponge cake with cream filling was a toxin produced by isolated staphylococci *(17).* The growth and proliferation of enterotoxigenic *Staphylococcus aureus* and other species with subsequent production of enterotoxin in foods presents a potential hazard to consumer health. Some of the reasons for examining suspect foods are (i) to confirm that the isolated organism is the etiological agent of a foodborne outbreak, whether unintentional or intentional; (ii) to determine whether a food or food ingredient is the source of enterotoxigenic staphylococci; and (iii) to demonstrate postprocessing contamination due to human contact with processed foods or exposure of the food to inadequately sanitized surfaces. Foods that are subjected to postprocess contamination with enterotoxigenic types of staphylococci represent a particularly significant hazard because of the probable absence of competitive organisms that would normally restrict the growth of staphylococci and thus the production of enterotoxin *(39).* Of the many metabolic products synthesized by staphylococci, the enterotoxins pose the greatest risk to consumer health. Illness associated with ingestion of enterotoxins is one of the most economically significant foodborne diseases in the United States, costing approximately $1.5 billion each year *(49).* These structurally related toxicologically similar proteins are produced most often by *S. aureus,* although *Staphylococcus intermedius* and *Staphylococcus hyicus* also are enterotoxigenic *(1). S. intermedius,* although usually considered a veterinary pathogen *(42, 47),* was isolated from a butter blend and margarine incriminated in an outbreak of food poisoning *(11, 37).* A coagulase-negative *Staphylococcus epidermidis* isolate was responsible for at least one outbreak of food poisoning *(19).* These incidents indicate the need for testing of bacterial species other than *S. aureus* for enterotoxigenicity when they are found in large numbers and for preformed toxins in foods incriminated in a food poisoning outbreak.

A wide variety of foods will support the growth of enterotoxigenic staphylococci. Foods may become contaminated during preparation in homes and food establishments, and toxin will form if these contaminated foods are subsequently mishandled prior to consumption. Foods that are frequently incriminated in food poisoning include meat and meat products, poultry and egg products, tuna, chicken, potato salad, macaroni, bakery products such as cream-filled pastries, cream pies and chocolate éclairs, sandwich fillings, milk, and dairy products. In processed foods, contamination may come from human, animal, or environmental sources *(15).* Therefore, the potential for enterotoxin formation is greater in foods exposed to temperatures that per-

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mit the growth of enterotoxigenic *S. aureus* and other species, especially in fermented meats and dairy products. Although the potential for growth of enterotoxigenic staphylococci is always present, it is only when improper fermentation (e.g., lactic acid starter failure) takes place that enterotoxin is produced *(39).* Although *S. aureus* is destroyed in food by thermal processing, the organism's presence usually indicates contamination by food handlers via the skin, nose, and mouth. This contamination may be introduced directly into foods by workers on the process line who have hand and arm lesions caused by enterotoxigenic *S. aureus.* Workers with respiratory infections and that cough or sneeze near food products can also contaminate these products. Contamination of processed foods may also occur when deposits of contaminated food collect on food contact surfaces. The occurrence of large numbers of *S. aureus* in processed food suggests that sanitation conditions, temperature control during food processing, or both are inadequate.

S. aureus may be present in raw food, especially in animal products, and may not be associated with human contamination. Animal hides, skins, and feathers may be responsible for staphylococcal contamination, which may or may not result from lesions or bruised tissue *(39).* Dressed animal carcasses are commonly contaminated with staphylococci, and such contamination often is unavoidable. Raw milk and unpasteurized dairy products may contain large numbers of *S. aureus,* which may be associated with staphylococcal mastitis in infected cattle. Keeping raw and processed foods separate to prevent cross-contamination is important for achieving a higher degree of food safety *(39).*

Although the staphylococci are considered mesophilic, some strains of *S. aureus* grow at temperatures as low as 6 to 7°C. However, *S. aureus* usually grows at temperatures between 7 and 47.8°C, with optimum growth at 35°C. The pH range for growth is between 4.5 and 9.3, with optimum growth between pH 7.0 and 7.5 *(40).* The staphylococci are unique in being able to grow under lower water activity (a_w) conditions than other nonhalophilic bacteria. Growth of staphylococci has been demonstrated at as low as 0.83 a_w under ideal conditions. These low a_w conditions are too low for growth of many competing organisms. Most strains of *S. aureus* are highly tolerant to salts and sugars and can grow over an a_w range of 0.83 to >0.99 *(15).*

The significance of *S. aureus* presence in foods should be evaluated carefully because not all staphylococci are enterotoxigenic. The presence of large numbers of staphylococci in foods is not sufficient cause to incriminate a specific food as the vehicle of food poisoning. The potential for causing food poisoning cannot be ascertained without demonstrating the actual presence of preformed enterotoxin in a suspect food; however, demonstration of enterotoxigenicity of food isolates is circumstantial evidence of enterotoxigenic staphylococcal contamination. The presence of large numbers of enterotoxigenic staphylococci suggests that further proliferation could result in the formation of preformed enterotoxin. Conversely, neither the absence of *S. aureus* nor the presence of small numbers of bacteria is complete assurance that a food is safe. Physical treatment such as heating of contaminated food may adversely affect *S. aureus,* resulting in a diminished population or even death of viable organisms, but the heat-stable toxin can remain biologically active and thus elicit symptoms of enterotoxin poisoning *(9, 10, 13).* To prevent food poisoning outbreaks, it is necessary to keep foods either refrigerated (<10°C) or hot (>45°C) to prevent proliferation of the organisms to such numbers (10^5 to 10^6 staphylococci per g) necessary for formation of detectable toxin *(15).*

GENERAL TOXIN CHARACTERISTICS

The staphylococcal enterotoxins are single-chain proteins with little or no nonprotein residues *(7).* They are antigenic, with molecular weights of 26,000 to 29,000, are composed of polypeptide chains of 230 to 239 amino acid residues with a disulfide loop near the middle of the molecule, and are rich in lysine, aspartic acid, and glutamic acids *(16).* They are neutral to basic hydroscopic proteins with pI values of 7.0 to 8.6, with the exception of staphylococcal enterotoxin G (SEG) and SEH, which have pI values of 5.6 to 5.7. They are resistant to proteolytic enzymes such as trypsin and pepsin, which makes it possible for them to travel through the digestive tract to the site of action. These toxins also are highly heat stable, making them a potential health hazard when they are present in retorted foods. Some of their other general properties or characteristics have been described elsewhere *(16, 17).* The various toxin serotypes (SEA through SEE, SEG, and SEH) are similar in composition and biological activity but are identified serologically as separate proteins because of differences in antigenicity *(22).*

TOXICOLOGICAL ACTIVITY OF STAPHYLOCOCCAL ENTEROTOXINS

The signs and symptoms of staphylococcal food poisoning occur when foods containing enterotoxin are ingested in reasonably small amounts (100 ng). However, death can occur if large amounts are ingested. Although the enterotoxins are not considered highly lethal because of the low mortality rate associated with the illness, they are considered a potential threat to health because of the characteristic human incapacitation. Fatality rates for cases that have been reported to public health authorities range from 0.03% in the general public to as high as 4.4% for highly sensitive populations such as children and elderly persons *(32).* The illness is acute, with onset occurring 1 to 7 h after ingestion of toxin-contaminated foods *(16, 25).* The early signs are nausea and possibly abdominal cramping, resulting in primary symptoms of vomiting and diarrhea. Secondary symptoms of the illness may include retching, sweating, headache, dehydration, marked prostration, muscular cramping, and a drop in blood pressure. Body temperature may be above or below normal. Blood and mucus may be observed in feces and vomitus in extreme cases *(18).* The complete effect of staphylococcal enterotoxin on humans has not been elucidated; however, some mechanisms have been proposed by a number of investigators *(17, 31).*

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Symptoms resulting from inhalation or intravenous administration of toxins differ from those occurring when enterotoxins are ingested, and this difference is related to the toxin's superantigenic properties. By binding directly to major histocompatibility complex molecules on the surface of antigen-presenting cells, the toxin stimulates proliferation of large numbers of T cells and the production and secretion of various cytokines from immune cells. These mitogenic activities have been proposed as major contributing factors for the development of shock in animals infected with enterotoxigenic staphylococci *(32).* Recovery from staphylococcal intoxication by ingestion generally occurs in 1 to 3 days. In contrast, victims infected via inhalation may require 1 to 2 weeks for complete recovery. The amount of enterotoxin that causes illness is not specifically known. Information from food poisoning outbreaks and human challenge studies *(18, 27, 33)* indicates that an enterotoxin dose of less than 1.0 μg in contaminated food will cause symptoms of staphylococcal intoxication. However, in highly sensitive people, a dose of 100 to 200 ng is sufficient to cause illness *(17).*

IDENTIFICATION OF ENTEROTOXINS

The identification of staphylococcal enterotoxins in foods is focused on two food types: foods that have been incriminated in food poisoning outbreaks and foods that are suspected of containing toxin because of time-temperature abuse or some other anomaly such as intentional contamination *(15).* In the former case, identification of enterotoxin presence supports the epidemiological findings that a specific food is responsible for a food poisoning outbreak or episode. In the latter case, the presence or absence of toxin will determine the marketability of the product. The isolation and determination of enterotoxigenicity of staphylococcal isolates in foods can serve as a signal for potential toxin formation if the product were to be further time-temperature abused, allowing for proliferation of the organism and the subsequent formation of preformed toxin should the populations of enterotoxigenic staphylococci rise to 10^6 CFU/g or greater. Determining the enterotoxigenicity of staphylococci by examining isolates for toxin production might be helpful in identifying the toxin serotype in a food incriminated in a food poisoning outbreak. Determination of the enterotoxigenicity of staphylococcal isolates is particularly important for demonstrating the prevalence of the various toxin serotype producers in foods and food-associated areas.

Although foods incriminated in food poisoning outbreaks generally contain large amounts of enterotoxin, occasionally these incriminated foods contain very small amounts of toxin, which makes identification more difficult. Enterotoxins can be readily identified from food products or other sources for which bacterial counts are or were at some time $\geq 10^6$ enterotoxigenic staphylococci per g of product. Such high bacterial counts in foods are unacceptable. Marketable foods should contain no enterotoxin, as determined by rapid and classical methods. The presence of enterotoxin in foods introduced into interstate consumer channels is in violation of the federal Food, Drug and Cosmetic Act.

Most food poisoning events result from eating foods in which enterotoxin has been produced after the foods are cooked or heated. However, some outbreaks result from eating foods that have been heated after enterotoxin has been produced *(16).* This problem can be particularly acute in the food processing industry, where heating steps are used in the production or preservation of the food product. A reasonably quick heat process such as pasteurization of milk will kill staphylococci but will not biologically inactivate the toxin that may have been produced prior to the pasteurization process *(48).* Other foods that receive sufficient heat to render the staphylococci nonviable and yet can cause food poisoning include boiled goat's milk *(30),* spray-dried milk *(4, 5),* cooked sausage *(48),* and canned lobster bisque *(23).* In 1982, thermally processed infant formula was incriminated in foodborne illnesses *(8),* and in 1989 canned mushrooms caused a number of outbreaks of staphylococcal intoxication *(9, 24).* In the food poisoning cases associated with the ingestion of toxin-contaminated mushrooms, the product was serologically negative but there was enough biologically active toxin to cause illness, as indicated by symptoms consistent with staphylococcal intoxication. To better understand the disparity between serological inactivity and human intoxication, studies were designed to better understand a lack of correlation between serological and biological activities of thermally stressed enterotoxin *(9, 10, 13).* The results of these studies indicated that the enterotoxin had been denatured, and as a consequence conformational changes in the molecule made it unrecognizable by the antibody developed by the native toxin protein. Methods were developed to renature the heated toxin *(9, 10, 13),* thus restoring the serological activity and making it possible to use serological methods for identification of previously heat-denatured enterotoxins. This approach to restoration of activity has since been applied by other investigators *(2, 3, 20, 50)* with varying degrees of success.

METHODS FOR ENTEROTOXIN IDENTIFICATION

Biological tests. Prior to the development of serological methods for the identification of the staphylococcal enterotoxins, all toxins were identified by observing emetic responses in young rhesus monkeys (*Macaca mulatta*) *(46).* In the monkey assay, the animal was observed up to 5 h after a suspect food extract was intubated to the stomach. If vomiting occurred during the observation period, the food extract was judged to contain toxin *(17).* Although this animal assay is considered specific, a number of disadvantages exist *(45).* An alternative bioassay involves intravenous injection of toxin in kittens *(28, 29, 35).* However, other bacterial metabolites can cause nonspecific emetic responses, although they can be either inactivated or neutralized *(45).* Both of these animals assays suffer from problems of expense, animal maintenance, varying degrees of sensitivity with different animals of the same species, and

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the presence of interfering substances *(40)*, which complicates proper interpretation of results.

Serological methods. The staphylococcal enterotoxins have been identified as serological entities and have been differentiated by serotype, making it possible for serological identification of the staphylococcal enterotoxins in the analytical laboratory. A number of methods involving either polyclonal and more recently monoclonal antibodies have been used to detect and measure these enterotoxins. Earlier methods utilized precipitation *(21)* and agglutination approaches, but more recent methods employ tracer-labeled or tagging methods to increase sensitivity *(40)*. Systems based on serological tools can in general be divided into a number of antigen-antibody reaction types: (i) gel immunodiffusion by direct precipitation or precipitation inhibition assays, (ii) agglutination assays, and (iii) tracer-labeled or tagged immunoassays. The most common of the earlier methods have been described in recent reviews *(17, 40, 45)*, and stepwise procedural details of selected enzyme-linked immunosorbent assay (ELISA) methods have been published in the *Bacteriological Analytical Manual (12)* and the *Compendium of Methods for the Microbiological Examination of Foods (39)*. Of the earlier methods, the microslide gel double-diffusion method has been approved by the AOAC International *(6)*. Su and Wong *(45)* provided an excellent review of the advantages and limitations in the use of classical and more advanced technologies for the identification of the staphylococcal enterotoxins.

ELISA-BASED METHODS FOR IDENTIFYING STAPHYLOCOCCAL ENTEROTOXINS

Tracer-labeled immunoassays have become highly popular in the last decade for identifying staphylococcal enterotoxins in foods and for determining the enterotoxigenicity of suspect staphylococci from foods and other sources. These tracer-type assays are binding assays in which the antigen-antibody reactions serve as the base or initial reaction and the enzyme or luminescent reactions serve as the marker. ELISA-based methods are of this type, in which the antibodies can be attached to a variety of solid supports such as paper disks, polyvinyl, or polystyrene and retain serological activity and specificity. The ELISA is classified as either homogeneous, in which the immunoreactants are dispersed in solution, or heterogeneous, in which the reactants are bound to a solid matrix *(40)*.

Several ELISAs have been proposed for the identification of the staphylococcal enterotoxins, and these assays currently are most commonly used because of their rapidity and sensitivity. The ELISA was first introduced by Saunders and Bartlett in 1977 for the detection of SEA in foods *(43)*. Since that time, a number of variations of ELISA-based methods have been introduced *(34, 36, 38, 41, 44)*. The assays used for the identification of enterotoxins have been either competitive or noncompetitive ELISAs *(36, 44)*. Enzyme conjugated with toxin competes with enterotoxin in samples for binding sites on immobilized antibodies. Binding of the enzyme-conjugated toxins to antibodies is dependent on the amount of toxin in the sample. The more toxin present in the sample, the less enzyme-conjugated toxin will bind the antibodies. The amount of toxin in the sample is determined by color development after addition of the substrate. The primary disadvantage to this type of assay is that large amounts of highly purified toxin are required. Of the competitive and noncompetitive ELISA-based methods, the noncompetitive double-antibody sandwich ELISA is the most popular *(33, 41)*. For this assay, specific antibodies are absorbed onto a solid support such as polystyrene balls, plastic microtiter wells, or plastic tubes. The antibody absorbed onto the solid support is the capture antibody. The enterotoxin in samples are bound to the capture antibodies and subsequently detected by the addition of an enzyme-labeled (conjugated) secondary antibody whose enzyme acts on a suitable substrate, producing a color reaction. The intensity of the color reaction is proportional to the amount of toxin in the sample. Absorbance data from each determination can be generated with a single- or dual-wavelength microtiter plate reader. Most of the ELISA-based methods are designed as polyvalent systems, which contain the anti-enterotoxin serotypes (SEA, SEB, $SEC_{1,2,3}$, SED, and SEE) or SEA-SED as a single detector utilized as a screening system, or have been developed as a monovalent system and thus are capable of differentiating among enterotoxin serotypes. Although both polyvalent and monovalent systems have their specific uses, some laboratories initially screen with a polyvalent system and then, if necessary, determine the specific serotype with a monovalent system. This approach is particularly helpful when analyzing a large number of samples and when toxin presence is rare compared with the large number of samples being analyzed.

Of the ELISA-based methods proposed for the identification of staphylococcal enterotoxins, only the TECRA-SET-VIA (TECRA International Pty. Ltd., Frenchs Forest, New South Wales, Australia) polyvalent ELISA has been evaluated exhaustively and approved by AOAC International *(14)*. Other double-antibody sandwich polyvalent ELISAs that have been proposed for the identification of toxins in foods include the Transia Tube SET and Transia Plate SET (Diffchamb AB, Västra Frölunda, Sweden), the Microtiter-Plate-SET (Brommeli, Bern, Switzerland), and the Vidas SET-II (bioMérieux, Marcy l'Etoile, France), an automated ELISA. Another, recently developed automated system for the detection of staphylococcal enterotoxin is the Unique Plus System (TECRA). A system that shows much promise as an automated approach for the detection of enterotoxin is the Vidas SET-II assay, which is an enzyme-linked fluorescent assay (ELFA). This immunoanalyzer is highly sensitive (0.25 to 1.0 ng/g) because of its fluorescent tag. It automatically completes all the ELISA steps and provides printout data and results in approximately 80 min, with an overall assay time of 1.5 h compared with approximately 4 h for the manual TECRA-SET. Other ELISA-based methods are monovalently configured and dedicated to identifying the specific serotypes of enterotoxin in foods but employ a single capture antibody and single antibody-enzyme conjugate for each serotype. The SET-EIA polystyrene ball method (Diagnostiche Labora-

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TABLE 1. *Comparison of three polyvalent ELISA-based methods for the identification of staphylococcal enterotoxins (≤1 ng/g) in foods*

Sample designation	Food product	Enterotoxin (A–D)	ELISA response		
			TECRA (absorbance value)[a]	VIDAS (test value)[b]	TRANSIA, Tube (absorbance value)[c]
501	Beef/pasta	SEB	+ (0.223)	+ (0.22)	+ (0.905)
1500	Infant formula	SEA	+ (0.272)	+ (0.23)	+ (0.306)
2300	Water chestnuts	SED	+ (0.544)	+ (0.31)	+ (0.919)
S-62/64	Cheese	SEC	+ (0.541)	+ (0.38)	+ (0.422)
62/1900	Cheese	SEA	− (0.137)	+ (0.22)	− (0.241)
1700/1800	Beef/pasta	SEA	+ (0.317)	+ (0.24)	+ (0.283)
2100	Rotini (pasta)	SEA	+ (0.237)	+ (0.29)	+ (0.359)
901	Chicken	SEA	+ (0.215)	+ (0.16)	− (0.041)

[a] Absorbance values were determined on a dual-wavelength microtiter plate reader at 405 and 492 nm. Values >0.200 indicate toxin presence.
[b] Test value is the quantity calculated according to the analytical method. Values >0.13 indicate toxin presence.
[c] Photometric readings for analytes were determined at 450 nm. Values >0.25 indicate analyte presence.

tories, Bern, Switzerland) and the Ridascreen-SET (R. Biopharm Gmbh, Darmstadt, Germany) are examples of this approach. TECRA has taken a rather unique approach by developing a monovalent ELISA (TECRA-SET-ID) that utilizes a single specific serotype antibody as the capture antibody with polyvalent secondary antibodies (anti-A, -B, -C, -D, and -E) conjugated to the enzyme instead of each secondary antibody conjugated separately to the enzyme. An exhaustive review of the advantages and limitations of these methods was presented by Su and Wong *(45)*. A comparison of the specificities and relative sensitivities of three ELISA-based methods (TECRA-SET VIA, VIDAS SET assay, and Transia Tube SET) for the identification of enterotoxin in a variety of foods is presented in Tables 1 through 4. A single food extraction procedure (TECRA) was applied to eliminate extraction as an additional parameter for consideration. Naturally contaminated canned mushrooms containing SEA were chemically treated *(10)* to restore the serological activity of the toxin prior to serological analysis of the mushroom extracts for enterotoxin presence. In foods to which enterotoxins were added (Table 1), the automated Vidas system identified toxin in all of the foods studied, but the TECRA ELISA and the Transia Tube ELISA failed to identify SEA in cheese. These data indicate that the Vidas system is slightly more sensitive than the other two assays. The TECRA ELISA and Vidas assay identified SEA in chicken, but the Transia ELISA did not. Two of the methods evaluated (TECRA and Vidas) were equally specific (Table 2), i.e., they produced negative results for foods that did not contain toxin. The Transia system produced a false-positive reaction only with chestnuts. All the methods studied were effective in the detection of toxin in foods

TABLE 2. *Specificity of three ELISA-based methods for the identification of staphylococcal enterotoxin in foods not containing toxins*

Sample designation	Food product	ELISA response		
		TECRA (absorbance value)[a]	VIDAS (test value)[b]	TRANSIA, Tube (absorbance value)[c]
201	Mushrooms	− (0.104)	− (0.03)	− (0.042)
1400	Nonfat milk	− (0.106)	− (0.02)	− (0.041)
601	Beef/pasta	− (0.092)	− (0.03)	− (0.051)
1600	Infant formula	− (0.114)	− (0.03)	− (0.041)
2400	Water chestnuts	− (0.175)	− (0.07)	+ (0.237)
S-62	Cheese	− (0.059)	− (0.02)	− (0.063)
S-10	Eggs	− (0.065)	− (0.03)	− (0.085)
1800	Beef pasta	− (0.058)	− (0.04)	− (0.070)
401/405	Milk	− (0.059)	− (0.03)	− (0.058)
2200	Rotini (pasta)	− (0.067)	− (0.04)	− (0.058)
S-12	Beef/pasta	− (0.071)	− (0.04)	− (0.060)
1200	Mushrooms	− (0.075)	− (0.03)	− (0.058)
1001/1005	Chicken	− (0.074)	− (0.03)	− (0.067)

[a] Absorbance values were determined on a dual-wavelength microtiter plate reader at 405 and 492 nm. Values ≤0.200 indicate no enterotoxin found.
[b] Test value is the quantity calculated according to the analytical method. Values >0.13 indicate enterotoxin presence.
[c] Photometric readings for analytes were measured at 450 nm. Values >0.25 indicate enterotoxin presence.

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TABLE 3. *Identification of staphylococcal enterotoxin by three ELISA-based methods in foods incriminated in food poisoning outbreaks*

Sample designation	Food product	ELISA response		
		TECRA (absorbance value)[a]	VIDAS (test value)[b]	TRANSIA, Tube (absorbance value)[c]
029-A	Turkey	+ (0.371)	+ (0.18)	+ (0.703)
029-B	Ham	+ (2.451)	+ (1.44)	+ (ND)[d]
731-9	Bar-b-que	+ (0.600)	+ (1.16)	+ (0.400)
FP-51	Taco salad	+ (>3.00)	ND	+ (0.711)
FP-67	Poi[e]	+ (0.835)	ND	+ (0.885)
060	Coconut cream pie	+ (0.994)	+ (1.44)	ND

[a] Absorbance values were determined on a dual-wavelength microtiter plate reader at 405 and 492 nm. Values >0.200 indicate toxin presence.
[b] Test value is the quantity calculated according to the analytical method. Values >0.13 indicate toxin presence.
[c] Photometric readings for analytes were measured at 450 nm. Values >0.25 indicate toxin presence.
[d] ND, not determined.
[e] Starch product (taro) similar to potatoes.

implicated in food poisoning outbreaks (Table 3). The Vidas system (ELFA) was the most efficient for identification of the renatured toxin in canned mushrooms (Table 4), followed by the TECRA ELISA and the Transia Tube ELISA.

Significant progress has been made in the development of serological methods for the identification of staphylococcal enterotoxins. However, these methodologies must be validated as rapid, sensitive, and specific approaches for the monitoring of foods for intentional and unintentional contamination with this microbial toxin.

TABLE 4. *Evaluation of three ELISA-based methods for the serological identification of heat-inactivated, chemically reactivated staphylococcal enterotoxin A in naturally contaminated canned mushrooms*

Sample designation	ELISA response		
	TECRA (absorbance value)[a]	VIDAS (test value)[b]	TRANSIA, Tube (absorbance value)[c]
170-1A	+ (0.530)	+ (0.48)	+ (0.850)
171-1A	+ (0.422)	+ (0.28)	+ (0.422)
172-15A	+ (1.503)	ND[d]	+ (0.617)
173-21A	+ (0.687)	ND	+ (1.228)
249-6	+ (0.312)	+ (1.97)	ND
311-1	+ (0.468)	ND	+ (>1.029)
059-4	+ (0.233)	+ (0.27)	+ (0.248)
059D-13	+ (0.264)	+ (0.84)	+ (0.330)
059D-15	+ (0.224)	+ (0.49)	− (0.158)
059D-16	+ (0.382)	+ (0.55)	− (0.196)
059D-17	+ (0.207)	+ (0.43)	− (0.163)
059D-18	− (0.170)	+ (0.14)	− (0.138)

[a] Absorbance values were determined on a dual-wavelength microtiter plate reader at 405 and 492 nm. Values >0.200 indicate toxin presence.
[b] Test value is the quantity calculated according to the analytical method. Values >0.13 indicate toxin presence.
[c] Photometric readings for analytes were measured at 450 nm. Values >0.25 indicate toxin presence; those <0.25 indicate undetectable toxin.
[d] ND, not determined.

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