J. Hyg., Camb. (1985), 95, 131–138
Printed in Great Britain

The use of a sandwich ELISA for the detection of staphylococcal enterotoxin A in foods from outbreaks of food poisoning

By ANTONNETTE A. WIENEKE and R. J. GILBERT
Food Hygiene Laboratory, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT

(*Received* 15 *January* 1985; *accepted* 21 *January* 1985)

SUMMARY

Foods from outbreaks of food poisoning were examined for the presence of staphylococcal enterotoxin A (SEA) by a sandwich ELISA using microtitre trays as the solid phase and SEA antibodies raised in sheep.

The presence of SEA was confirmed by neutralization tests. The toxin was detected in 12 of 15 foods from separate outbreaks of staphylococcal food poisoning; all 15 foods contained a strain of *Staphylococcus aureus* which produced SEA. For most foods a simple extraction procedure without a concentration step was sufficient to detect the toxin. The method was semi-quantitative and recoveries of SEA added to control foods varied from 30 to 80 %. The foods from outbreaks contained between 1 and 10 μg of SEA/100 g. SEA was not found in foods from 21 outbreaks in which an SEA-producing strain of *Staph. aureus* was not isolated.

INTRODUCTION

Staphylococcal food poisoning is caused by enterotoxins produced by strains of *Staphylococcus aureus* during their growth in contaminated food. At least five serologically distinct enterotoxins, designated A, B, C, D and E, are now recognized and all have been implicated in food poisoning outbreaks in this country. The majority of outbreaks are caused by strains producing enterotoxins A (50 %) or both A and D (21 %) (Gilbert & Wieneke, 1973; de Saxe, Coe & Wieneke, 1982).

A number of reports have been published on the detection of staphylococcal enterotoxins in foods by ELISA. Simon & Terplan (1977), Stiffler-Rosenberg & Fey (1978), Kauffman (1980) and Kuo & Silverman (1980) developed the competitive ELISA while Saunders & Bartlett (1977), Freed *et al.* (1982) and Notermans *et al.* (1983) developed the sandwich technique. Fey, Pfister & Rüegg (1984) compared four different test systems: competitive ELISA with labelled enterotoxin, sandwich ELISA with labelled antibody and inhibitory tests with labelled and unlabelled antibody. The sandwich ELISA was shown to be the most satisfactory method. Using polystyrene balls and 20 ml food extracts a sensitivity of 0·01 μg of enterotoxin/100 g of food was achieved. A diagnostic kit based on this method is now commercially available (Labor Dr W. Bommeli, Bern, Switzerland).

This paper describes the detection of enterotoxin A in foods from outbreaks of staphylococcal food poisoning using the slightly modified sandwich ELISA of Notermans *et al.* (1983). In some cases the results are compared with those from the gel diffusion method.

MATERIALS AND METHODS

Foods

The samples of food implicated in the outbreaks of food poisoning included in this study were received from Public Health and hospital laboratories in the U.K. from June 1981 to August 1984. Foods used for the construction of standard curves and for recovery experiments were purchased from local shops.

Enterotoxins and antisera

Enterotoxins A (SEA) and B (SEB) were provided by Professor J. Melling, Porton, U.K. and by Professor M. S. Bergdoll, Wisconsin, U.S.A. Professor Bergdoll also donated rabbit anti-SEA and anti-SEB serum. Sheep immunoglobulins (IgG), anti-SEA and anti-SEB and their conjugates with horseradish peroxidase were obtained from Dr S. Notermans, Bilthoven, Netherlands. Rabbit antisera to SEA and SEB used for neutralization tests were prepared using the procedure of Bradstreet *et al.* (1977).

Food extraction

The amount of food sent for enterotoxin tests varied from 0·5 g to more than 100 g. For the enterotoxin extraction the whole sample, but not more than 100 g, was extracted by the method of Reiser, Conaway & Bergdoll (1974). The food was homogenized with an equal amount of sterile distilled water to form a slurry. For dried foods such as lasagne twice the amount of water was used. Small samples of food (less than 1 g) were also extracted with a larger volume of water, and the extract was concentrated using polyethylene glycol (PEG). After adjusting the pH to 4·5 with 1 N-HCl the homogenized food slurry was centrifuged for 20 min at 15000 ***g***. The solid fat layer was discarded, the supernatant fluid removed and the residue extracted again with half the amount of distilled water used originally and centrifuged. The second procedure was carried out to extract as much enterotoxin as possible for the gel-diffusion enterotoxin-detection technique. The supernatant fluids obtained from the two extraction steps were combined and the pH adjusted to 7·0 with 5 N-NaOH. If a precipitate formed, the extract was centrifuged again. The supernatant fluid was shaken with 20% of its volume of chloroform and centrifuged at 2500 ***g*** for 10 min. The aqueous layer was used in the ELISA both directly and after concentration in PEG. Most food extracts became too viscous to pipette when concentrated more than about 10 times.

Recovery experiments

SEA (0·1–20 μg/100 g) was added to 10 or 20 g samples of food. The samples were extracted as described, but the second extraction step was omitted. Foods used in these experiments were boiled ham (2 tests), canned salmon (3), boiled egg with mayonnaise or bread (2) and chicken and ham paste from a jar, dried lasagne and meat pie (1 test each).

For the detection of enterotoxin by the double gel-diffusion slide test the food extract needed further treatment (Casman, 1967; Gilbert *et al.* 1972). The final concentrated food extract was diluted to about 20 ml with 0·01 M phosphate buffer, pH 7·4 and shaken with 20% of its volume of chloroform. After centrifugation at

2500 *g* for 10 min, the water layer was further diluted with 40 volumes of 0·005 M phosphate buffer, pH 5·7. If necessary the pH was adjusted to 5·7. The diluted extract was then passed through a carboxymethylcellulose (CM 32, Whatman Ltd, England) column (5–10 g wet weight equilibrated in the dilution buffer). After washing the column with 100–200 ml of the dilution buffer, the absorbed toxin was extracted with 100–200 ml of 0·2 M phosphate buffer in saline, pH 7·4. The eluate was dialysed against distilled water for 2 h, concentrated to about 5 ml using PEG and then shaken with 20 % of its volume of chloroform. If after centrifugation the water layer was not clear, the chloroform treatment was repeated. The water layer was concentrated to a final volume of 0·2 ml and tested for the presence of enterotoxin by gel diffusion (Crowle, 1958; Šimkovičová & Gilbert, 1971).

The ELISA

The sandwich technique of Notermans *et al.* (1983) was used with minor modifications. Wells of microtitre trays (Dynatech M 129 B) were coated with antibody by adding 0·1 ml of sheep anti-staphylococcal enterotoxin–(anti-SE)–IgG in 0·07 M phosphate buffer in saline pH 7·2 (PBS) to each well. After overnight incubation at 22 °C the wells were washed four times with 0·05 % Tween 20 in distilled water using an Ilacon plate washer (Ilacon, Tonbridge, Kent). After washing, 0·1 ml volumes of the test samples were added to the wells. The test samples were diluted 1:2 with PBS which contained 0·1 % Tween 20 and 0·2 % normal rabbit serum or 0·2 % homologous anti-SE serum for confirmation of the presence of enterotoxin (SE) (Duermeyer, 1980). In preliminary experiments it was determined that a final concentration of 0·01 % anti-SEA serum could still neutralize all SEA in the samples, but 0·001 % could not. The trays were incubated for 90 min at 22 °C. After washing as previously described, 0·1 ml volumes of anti-SE IgG–horseradish peroxidase conjugate in PBS containing 0·05 % Tween 20 were added to the wells. The trays were incubated at 22 °C for 90 min, washed again and 0·1 ml volumes of substrate solution [4 mg 2,2'-azino-di-(3-ethylbenzthiazoline sulphonate) (ABTS) (Sigma, London) in 10 ml citrate–phosphate buffer, pH 4, with 0·064 ml 6 % H_2O_2 added just before use] were added to each well. After incubation at 22 °C for 30 min, light absorption was measured at 405 nm with a microtitre plate reader. The concentrations of anti-SE-IgG used for coating and of IgG-enzyme conjugate were determined by chequerboard titration.

A sample was considered to contain SE when the absorbance at 405 nm of the sample was normal rabbit serum was at least twice that of the sample with the homologous anti-SE serum.

RESULTS

Recovery experiments

The recovery of SEA from foods to which known amounts of SEA were added was estimated by using standard curves of SEA in control food extracts and by assuming an equal distribution of enterotoxin between the liquid and solid phase of the homogenized food. When SEA was added to samples of food 30–80 % could be recovered (Table 1). Results varied up to two-fold from experiment to experiment. The smallest amount of SEA that could be detected after the

Table 1. *Recovery of enterotoxin A added to foods*

Food	Recovery (%) of SEA added to foods at various concentrations (μg/100 g)*						
	0·1	0·2	0·5	1·0	2·0	10·0	20·0
Ham	−†	+	NT	40	50	60	60
Salmon (canned)	−	+	+	50	60	70	80
Chicken and ham paste (jar)	NT	−	−	30	40	50	NT
Dried lasagne	NT	+	+	50	50	NT	NT
Boiled egg with mayonnaise or bread	NT	+	NT	NT	60	NT	NT
Meat pie	NT	+	NT	NT	40	NT	NT

NT, not tested.

* Tests were carried out on concentrated food extracts when less than 1·0 μg of SEA/100 g was added and on unconcentrated extracts when 1·0 μg of SEA or a larger amount was added.

† The concentrated extracts were viscous and no attempt was made to quantify the results.

−, SEA not found; +, SEA present.

Table 2. *Detection of enterotoxin A by ELISA in foods from outbreaks of staphylococcal food poisoning*

Outbreak number	Food	Count of *Staph. aureus*/g food	Enterotoxin(s) produced by strain	Presence of enterotoxin A in the food measured by	
				ELISA	Gel diffusion
1	Ham	$1·5 \times 10^9$	A	+	NT
2	Vanilla slice	$1·3 \times 10^9$	A	+	+
3	Ham roll	$1·2 \times 10^9$	A, D	+	+
4	Salmon mousse	9×10^8	A, D	+	NT
5	Egg sandwich	7×10^8	A	+	+
6	Ham	5×10^8	A, D	+	+
7	Lasagne (dried)	2×10^8	A	+	−
8	Fried chicken spread (jar)	9×10^7	A	+	NT
9	Chicken and ham paste (jar)	5×10^7	A, B, D	+	−
10	Ham	$1·5 \times 10^7$	A, D	+	NT
11	Pizza	4×10^6	A	+	NT
12	Salmon (canned)	$3·5 \times 10^6$	A	+	−
13	Chicken	1×10^5	A	−	−
14	Salmon (canned)	2×10^4	A	−	−
15	Fish fingers	2×10^4	A	−	NT

NT, not tested.

extraction procedure was about 1 μg/100 g in the unconcentrated extract and about 0·2 μg/100 g in the concentrated extract.

Detection of SEA in foods from outbreaks

Foods from 15 separate outbreaks of staphylococcal food poisoning were tested for the presence of SEA by ELISA. In all cases *Staph. aureus* was isolated from the food, mostly at levels $> 10^6$ *Staph. aureus*/g. These strains produced SEA,

https://doi.org/10.1017/S0022172400062367 Published online by Cambridge University Press

Table 3. *Detection of enterotoxin A by ELISA in foods from outbreaks of possible staphylococcal food poisoning*

	Number of outbreaks	Number of foods with detectable SEA
Outbreaks caused by SEA	15	12
'Other' outbreaks*	21	0

* Outbreaks in which *Staph. aureus* could not be isolated from the food (9); outbreaks in which the isolated *Staph. aureus* strain did not produce one of the recognized enterotoxins (8); outbreaks caused by enterotoxins other than SEA (4).

sometimes together with other enterotoxins (Table 2). SEA was detected in the unconcentrated extract of nine foods. The ratio of the absorbance at 405 nm of the sample incubated with normal rabbit serum (N) and of that incubated with anti-SEA serum (P) was greater than 4 in all cases and greater than 7 in seven samples. The absorbance at 405 nm of the samples with anti-SEA serum served as blanks and varied from 0·06 to 0·20 for the different kinds of food. In one outbreak only 0·5 g salmon mousse was available for testing. It was extracted with 5 ml water and the extract concentrated 10 times. SEA was detected in the concentrated extract with an N/P ratio of greater than 10. The N/P ratio of two foods (ham $1{\cdot}5 \times 10^7$ *Staph. aureus*/g, pizza 4×10^6 *Staph. aureus*/g) was about 2 for the concentrated extracts. These two extracts were further concentrated as described for the gel diffusion method, and the presence of a small amount of SEA was confirmed by testing the final extract of about 1 ml by ELISA. SEA was not found in the concentrated extracts of three foods, all of which contained $< 10^6$ *Staph. aureus*/g.

SEA was not detected by the double gel-diffusion slide method in 3 of 7 positive foods tested. One of the three foods was dried lasagne (2×10^8 *Staph. aureus*/g), and although a 100 g sample was extracted, the final extract gave an opaque ring round the well on the gel diffusion slide obscuring the results. Only small samples of the two remaining foods were available for testing; both had relatively small numbers of *Staph. aureus*, and a positive result was not expected.

One further outbreak was caused by SEB present in cooked pork. The food contained 6×10^9 *Staph. aureus*/g and SEB was detected by ELISA in the unconcentrated extract and also by the gel diffusion method.

Samples of food from outbreaks caused by enterotoxins other than SEA and samples of food from outbreaks of diarrhoea and vomiting in which either the *Staph. aureus* strain from the food did not produce one of the recognized enterotoxins or *Staph. aureus* could not be detected in the food were also examined for the presence of SEA by ELISA: all foods from the 21 outbreaks studied were negative (Table 3).

DISCUSSION

The required detection level of enterotoxin in a sample of food is 0·1–0·2 μg of enterotoxin/100 g of food (Bergdoll, 1979). Freed *et al.* (1982) could detect 0·1 ng of enterotoxin/ml in buffer using a sandwich ELISA with polystyrene balls as solid phase and 0·025–0·063 μg of enterotoxin/100 g of food, depending on the kind of food. Fey, Pfister & Rüegg (1984) were able to detect 0·01 μg/100 g of food using

a similar method. Notermans *et al.* (1983) detected 0·3 μg/100 g of food using a sandwich ELISA with microtitre plates as solid phase. The ball system is more sensitive than the microtitre plate system but less easily carried out (Freed *et al.* 1982), and in our experience the latter was sufficiently sensitive to detect enterotoxin in foods from most outbreaks. In this study the limit of detection of enterotoxin A in buffer using the sandwich ELISA with microtitre plates was about 1 ng/ml. Enterotoxin A was detected in 12 of 15 foods from separate outbreaks of staphylococcal food poisoning; enterotoxin B was detected in one sample of food from an additional outbreak. For most foods only a simple extraction procedure without a concentration step was necessary to prepare the sample for the test. The method was semi-quantitative, the foods containing between 1 and 10 μg of enterotoxin/100 g, which is within the range of concentrations found by Gilbert *et al.* (1972). Recovery experiments showed that the limit of detection was about 1 μg/100 g of food, and this could be lowered to about 0·2 μg/100 g by concentrating the extract. Non-specific reactions were not found; 21 foods from outbreaks in which no SEA-producing strain was isolated were all negative for SEA.

The non-interference of protein A in the ELISA by using antibodies raised in sheep (Notermans, Timmermans & Nagel, 1982) was confirmed by testing protein A at a concentration of 50 μg/ml. When using antibodies raised in rabbit Fey *et al.* (1984) eliminated protein A interference by adding 2·5–10% normal rabbit serum to the food extracts.

Using the extraction method of Casman (1967) and the micro slide gel diffusion test, SEA was detected in 59 of 87 (68%) foods from outbreaks of food poisoning caused by SEA (updated information from de Saxe, Coe & Wieneke, 1982). Sixteen of the negative foods contained $> 10^6$ *Staph. aureus*/g, and these would probably have given a positive result with the ELISA.

The limit of detection of the gel diffusion method is about 0·5 μg in any amount of food up to 100 g. The main advantage of the ELISA over the gel diffusion technique is the greater sensitivity and speed of the test: the enterotoxin can be detected in the unconcentrated extract, making it possible to obtain a result within 2 days, and it can be detected in small samples of food, as little as 0·5 g. In the gel diffusion method the toxin extraction from the food together with the incubation of the gel slides takes at least 5 days, and the method is not sufficiently sensitive to detect toxin in small samples of food.

The presence of SE in the samples was confirmed by neutralization of the toxin with anti-SE serum (Duermeyer, 1980). Fey *et al.* (1984) used balls coated with normal rabbit serum in parallel with balls coated with anti-SE serum to check for specificity.

To determine the amount of enterotoxin in a sample of food, Freed *et al.* (1982) advised that a standard curve in an extract of a control food as closely as possible resembling the test food should be prepared. Also, the loss of enterotoxin during the extraction procedure should be determined by checking the recovery of enterotoxin added to the control food before extraction.

Büning-Pfaue, Timmermans & Notermans (1981) and Notermans *et al.* (1983) found that standard enterotoxin curves in minced meat and pasteurized vanilla custard extracts were similar to those in buffer. In this study absorbance at 405 nm of standard SEA in food extracts was about 50% higher than that of SEA in phosphate buffer.

Büning-Pfaue, *et al.* (1981) detected 40% of 0·1 μg of SEB added to 100 g of pasteurized vanilla custard, and Freed *et al.* (1982) obtained recoveries of 50–80%, depending on the kind of food, when adding 0·06–0·125 μg of enterotoxins A to E/100 g of food. Notermans *et al.* (1983) recovered 40–80% of enterotoxins A, B, C and E added to minced meat samples.

In this study recovery of added SEA (0·2–20 μg/100 g) from a variety of foods ranged from 30 to 80%. For the micro slide gel diffusion test the recovery of enterotoxin is 20% (Gilbert *et al.* 1972). Because the ELISA is semi-quantitative, we did not determine standard curves in food extracts and carry out recovery experiments in all cases. Standard curves in different food extracts were similar, and added SEA was recovered from all foods tested. It is often difficult to obtain a food identical to the outbreak food for control tests. The control food was not needed to establish a blank value, because the absorbance at 405 nm of the sample incubated with anti-SE serum served as blank.

SEA was not found in three foods from outbreaks, although the strains isolated from the foods produced SEA. All three foods contained relatively low numbers of *Staph. aureus*. The failure to detect enterotoxin in these foods may have been due to the fact that either these foods were not part of the incriminated samples or that the staphylococci and enterotoxin were unevenly distributed throughout the foods.

Recently Igarashi *et al.* (1984) developed a reversed passive latex agglutination technique with a sensitivity of 1 ng/ml for the detection of enterotoxins in food. With this method they detected enterotoxin in foods from 26 outbreaks. A diagnostic kit based on their method for enterotoxins A, B and C is available in Japan (Denka Seiken Co. Ltd, Tokyo, Japan). This seems an even simpler method than the ELISA, and an evaluation of this method in comparison with the ELISA and the gel diffusion technique is necessary.

The authors wish to thank the Directors and staff of the Public Health and hospital laboratories for sending the samples of food and Professor M. S. Bergdoll, Food Research Institute, University of Wisconsin, U.S.A., Professor J. Melling, Centre for Applied Microbiology and Research, Porton Down, and Dr S. Notermans, Rijksinstituut voor Volksgezondheid en Milieuhygiene, Bilthoven, Netherlands, for their gifts of various reagents.

REFERENCES

Bergdoll, M. S. (1979). Staphylococcal intoxications. In *Food-Borne Infections and Intoxications*, 2nd ed. (ed. H. Riemann and F. L. Bryan), pp. 443–494. New York: Academic Press.

Bradstreet, C. M. P., Dighero, M. W., Gilbert, R. J. & Wieneke, A. A. (1977). Raising antibodies to staphylococcal enterotoxins A and B. *Journal of Applied Bacteriology* **42**, 117–122.

Büning-Pfaue, H., Timmermans, P. & Notermans, S.(1981). Einfache Methode für den Nachweis von Staphylokokken-Enterotoxin-B in Vanillepudding mittels ELISA – Test. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung* **173**, 351–355.

Casman, E. P. (1967). Staphylococcal food poisoning. *Health Laboratory Science* **4**, 199–206.

Crowle, A. J. (1958). A simplified micro double-diffusion agar precipitin technique. *Journal of Laboratory and Clinical Medicine* **52**, 784–787.

de Saxe, M., Coe, A. W. & Wieneke, A. A. (1982). The use of phage typing in the investigation of food poisoning caused by *Staphylococcus aureus* enterotoxins. In *Isolation and Identification Methods for Food Poisoning Organisms*. Society for Applied Bacteriology Technical Series

No. 17 (ed. J. E. L. Corry, D. Roberts and F. A. Skinner) pp. 173–197. London: Academic Press.

DUERMEYER, W. (1980). *Application of ELISA for the Diagnosis and Epidemiology of Hepatitis A*, pp. 85–86. (Thesis, University of Utrecht). Amsterdam: Rodopi.

FEY, H., PFISTER, H. & RÜEGG, O. (1984). Comparative evaluation of different enzyme-linked immunosorbent assay systems for the detection of staphylococcal enterotoxins A, B, C and D. *Journal of Clinical Microbiology* **19**, 34–38.

FREED, R. C., EVENSON, M. L., REISER, R. F. & BERGDOLL, M. S. (1982). Enzyme-linked immunosorbent assay for detection of staphylococcal enterotoxins in foods. *Applied and Environmental Microbiology* **44**, 1349–1355.

GILBERT, R. J. & WIENEKE, A. A. (1973). Staphylococcal food poisoning with special reference to the detection of enterotoxin in food. In *The Microbiological Safety of Food* (ed. B. C. Hobbs and J. H. B. Christian), pp. 273–285. London: Academic Press.

GILBERT, R. J., WIENEKE, A. A., LANSER, J. & ŠIMKOVIČOVÁ, M. (1972). Serological detection of enterotoxin in foods implicated in staphylococcal food poisoning. Journal of Hygiene **70**, 755–762.

IGARASHI, H., SHINGAKI, M., FUJIKAWA, H., USHIODA, H. & TERAYAMA, T. (1984). Detection of staphylococcal enterotoxins in food poisoning outbreaks by reversed passive latex agglutination. In *Proceedings of 5th International Symposium on Staphylococci and Staphylococcal Infections*. Warsaw.

KAUFFMAN, P. E. (1980). Enzyme immunoassay for staphylococcal enterotoxin A. *Journal of the Association of Official Analytical Chemists* **63**, 1138–1143.

KUO, J. K. S. & SILVERMAN, G. J. (1980). Application of enzyme-linked immunosorbent assay for detection of staphylococcal enterotoxins in food. *Journal of Food Protection* **43**, 404–407.

NOTERMANS, S., TIMMERMANS, P. & NAGEL, J. (1982). Interaction of staphylococcal protein A in enzyme-linked immunosorbent assays for detecting staphylococcal antigens. *Journal of Immunological Methods* **55**, 35–41.

NOTERMANS, S., BOOT, R., TIPS, P. D. & DE NOOY, M. P. (1983). Extraction of staphylococcal enterotoxins (SE) from minced meat and subsequent detection of SE with enzyme-linked immunosorbent assay (ELISA). *Journal of Food Protection* **46**, 238–241.

REISER, R., CONAWAY, D. & BERGDOLL, M. S. (1974). Detection of staphylococcal enterotoxin in foods. *Applied Microbiology* **27**, 83–85.

SAUNDERS, G. C. & BARTLETT, M. L. (1977). Double-antibody solid-phase enzyme immunoassay for the detection of staphylococcal enterotoxin A. *Applied and Environmental Microbiology* **34**, 518–522.

ŠIMKOVIČOVÁ, M. & GILBERT, R. J. (1971). Serological detection of enterotoxin from food-poisoning strains of *Staphylococcus aureus*. *Journal of Medical Microbiology* **4**, 19–30.

SIMON, E. & TERPLAN, G. (1977). Nachweis von Staphylokokken Enterotoxin B mittels ELISA-Test. *Zentralblatt für Veterinärmedizin* Reihe B **24**, 842–844.

STIFFLER-ROSENBERG, G. & FEY, H. (1978). Simple assay for staphylococcal enterotoxins A, B and C: modification of enzyme-linked immunosorbent assay. *Journal of Clinical Microbiology* **8**, 473–479.