APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 2005, p. 5590–5592 Vol. 71, No. 9
0099-2240/05/$08.00+0 doi:10.1128/AEM.71.9.5590–5592.2005

Biosensor Detection of Botulinum Toxoid A and Staphylococcal Enterotoxin B in Food

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Received 4 November 2004/Accepted 29 March 2005

Immunoassays were developed for the simultaneous detection of staphylococcal enterotoxin B and botulinum toxoid A in buffer, with limits of detection of 0.1 ng/ml and 20 ng/ml, respectively. The toxins were also spiked and measured in a variety of food samples, including canned tomatoes, sweet corn, green beans, mushrooms, and tuna.

The toxins staphylococcal enterotoxin B (SEB) and botulinum toxin A are responsible for food poisoning and have the potential to be used as biological warfare agents, with the current toxic dose for aerosol forms at 0.02 μg/kg of body weight and 0.07 μg/kg, respectively (3, 4, 28, 29). There is need for a rapid method of monitoring food, water, and air samples for both natural and intentional contamination by these toxins. Here, we demonstrate the rapid, simultaneous dose-dependent detection of SEB and botulinum toxoid A (BotA), as measured using the Naval Research Laboratory array biosensor (7). The array biosensor has successfully been used for the detection of a variety of species, initially in buffer but increasingly in food and environmental matrices (16–22, 24, 26, 27).

Here, biotinylated capture antibodies (10 μg ml^{-1} in phosphate-buffered saline containing 0.05% Tween 20 [PBST]) were patterned on NeutrAvidin-functionalized slides using a poly(dimethyl)siloxane flow cell, as described previously (16). Each slide was patterned with two columns specific for chicken immunoglobulin Y (positive controls), five specific for BotA, and five for SEB. Twenty-minute sandwich immunoassays were performed essentially as described previously (19, 22, 24) using a cocktail of fluorescently labeled tracer antibodies (100 ng/ml Cy5-chicken immunoglobulin Y and 10 μg/ml each of AlexaFluor647 anti-SEB and Cy5 anti-botulinum toxin A in PBST containing 1 mg/ml bovine serum albumin [PBSTB]) to detect bound targets (2, 24). Assays were developed with PBSTB for measurement of dose-response curves for both BotA and SEB simultaneously. The samples were spiked such that the concentrations of BotA decreased (0 to 500 ng/ml), while those of SEB increased (0 to 7.5 ng/ml). Four control samples were analyzed on each slide—two buffer controls containing only a single toxin (lanes 1 and 10) and an analogous set of spiked food matrices (lanes 2 and 9). A typical assay response is shown in Fig. 1, with a decreasing intensity of the BotA loci from top to bottom and increasing intensity for the SEB loci, corresponding to the concentration variation of the toxins. The different dynamic ranges used for the two toxins are a result of the different affinities of their respective antibodies. There is no apparent cross-reactivity between the species. The limit of detection (LOD), the lowest concentration giving 3 standard deviations above buffer-negative control values, was 20 ng/ml for BotA, although occasionally samples containing 5 ng/ml were positive by the same criteria. The SEB LOD was consistently found to be 0.1 ng/ml as previously reported (24). Although the LOD for BotA (20 ng/ml) is higher than that achieved by mouse bioassay, it is comparable to those of other rapid immunosensors (1, 4–6, 9, 11, 12, 14, 15, 18, 23, 25).

The sandwich assay format was used to detect the targets with which various food samples were spiked. Canned corn, green beans, and whole tomatoes were separated into liquid and solid components, and each component was tested separately. Only the solid components of canned tuna and canned mushrooms were tested. Each of the food solids was diluted 1:1 (wt/vol) with PBSTB and homogenized on high in a Waring Blender. Each sample was then spiked with 25× PBST and 250 mg/ml bovine serum albumin such that the final concentrations were 1× and 1 mg/ml, respectively; blended solid tomato samples were neutralized with NaOH. The samples were then spiked with a mixture of BotA and SEB to final concentrations of 1 to 500 ng/g and 0 to 7.5 ng/g, respectively. Liquid components from the canned corn, green beans, and tomatoes were treated similarly. All samples were mixed and left at room temperature for 2 h prior to analysis to allow the toxins to interact with the food matrix (24). The mixtures containing solids were then centrifuged at 3,000 rpm for 5 min, and the supernatant was collected and assayed.

The images collected for the various foods (e.g., Fig. 2A) were analyzed for the intensity of signal above background for each locus. To account for interslide variability and matrix effects, the dose-response curves were normalized using the spiked buffer controls (16, 17, 22, 27). Normalized dose-response curves for the spiked foods are shown in Fig. 2B and C, and the resulting LODs for all foods are summarized in Table 1. None of the unspiked foods tested caused false-positive results. Almost all of the food matrices studied caused a dampening of the BotA dose-response curve ($P < 0.01$) and a resulting increase in the LOD, in most cases, from 20 to 50 ng/ml.

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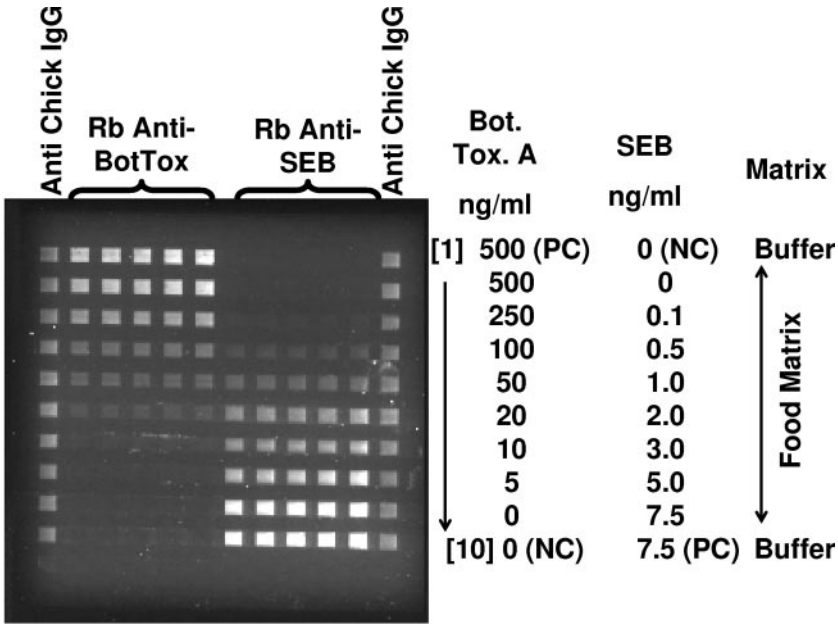

FIG. 1. Charge-coupled device (CCD) image of simultaneous 20-minute assays of samples containing BotA (0 to 500 ng/ml) and SEB (0 to 7.5 ng/ml) in PBSTB. Sample channel numbers are indicated by the numbers in brackets. Rb, rabbit; BotTox, botulinum toxin; IgG, immunoglobulin G; Bot. Tox. A, BotA; PC, positive control; NC, negative control.

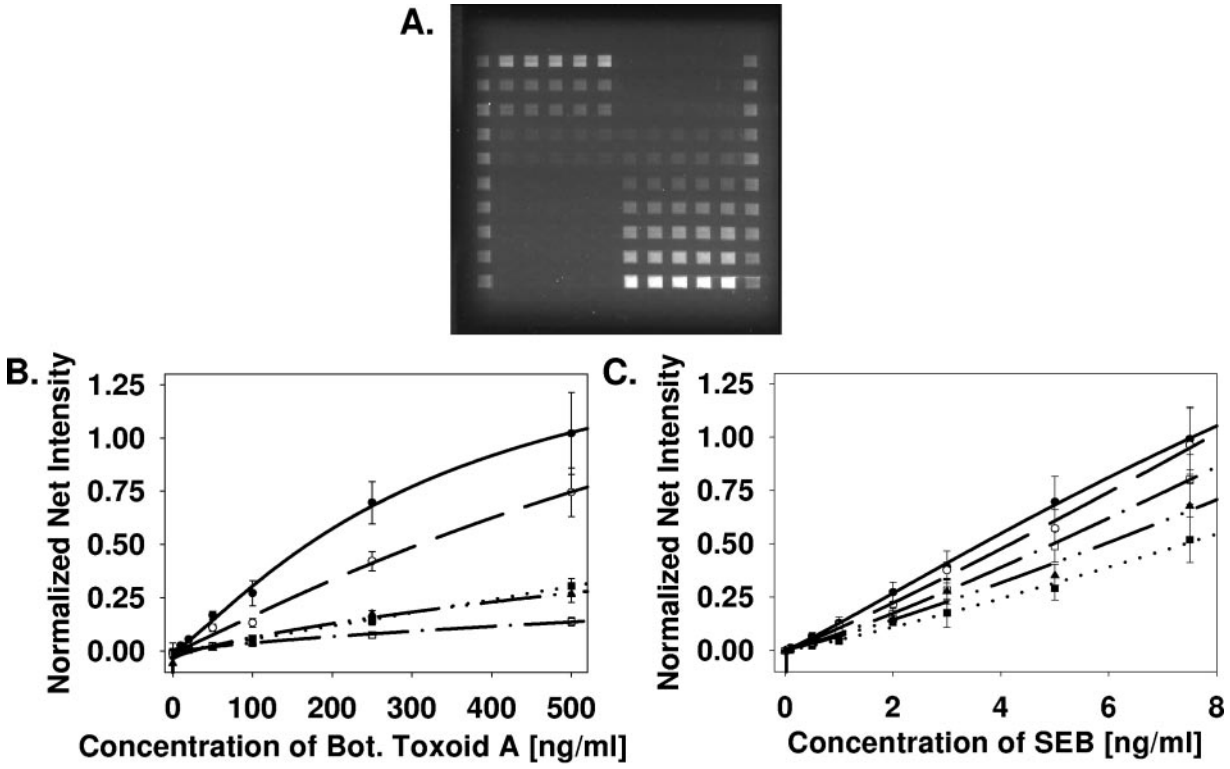

FIG. 2. (A) Final CCD image after analysis of spiked, unbuffered tomato juice. Positive and negative controls (PC and NC, respectively) are shown in lanes 1 and 10 as in Fig. 1. Dose-response curves for BotA and SEB in different food matrices, normalized to the appropriate spiked buffer control, are shown. The shorter exposure time was used to analyze SEB data because at longer exposures, the signal intensity reached the CCD maximum. (B) BotA in PBSTB (filled circles), buffered whole tomatoes (open circles), mushrooms (filled squares), green beans (open squares), and tuna (filled triangles). (C) SEB in PBSTB (filled circles), buffered whole tomatoes (open circles), mushrooms (filled squares), green beans (open squares), and tuna (filled triangles). A minimum of two replicate slides were run for each food sample, with each slide containing five replicates of the same data square. Shown are normalized mean net fluorescent intensities for 10 squares ± standard deviations. Bot., botulinum.

TABLE 1. Limits of detection obtained with different food matrices for BotA and SEB

Matrix	pH[a]	BotA LOD (ng/ml)[c]	SEB LOD (ng/ml)[c]
PBSTB	7.5	20	0.1
Tomato juice (unbuffered)	4.5	50	0.5
Tomato juice (buffered)	7.0	20	0.1
Whole tomatoes[b]	6.0	50	0.1
Whole tomatoes[b]	7.5	50	0.1
Mushrooms[b]	7.0	100	0.5
Sweet corn[b]	7.0	50	0.5
Sweet corn juice	7.0	50	0.5
Green beans[b]	7.0	250	0.5
Green bean juice	6.5	100	0.1
Tuna[b]	7.0	500	0.5

[a] The pH of the sample supernatant, after centrifugation, was measured using ColorpHaste (pH 2 to 9) paper (EMD Chemicals Inc., Gibbstown, NJ).

[b] These values are shown in nanograms per milliliter rather than nanograms per gram as for the solid foodstuffs to facilitate comparison between the various matrices and buffer samples, although they are equivalent.

[c] LODs were signals higher than 3 standard deviations above the LOD of the buffer-negative control.

Tuna and green beans, however, significantly inhibited the response, increasing the LOD to as much as 500 ng/ml for tuna ($P < 0.01$); these may require extraction or additional sample clean up to improve sensitivity. In contrast, the SEB immunoassay response was not as affected by the food matrix, with a maximum increase in the LOD from 0.1 to 0.5 ng/ml, still below the 1-ng/g goal for solution detection sought by USDA-FSIS (8, 10, 13, 16, 17, 24).

This study has demonstrated the ability of the Naval Research Laboratory array biosensor to detect BotA and SEB simultaneously in complex food matrices. The assays are simple to perform, show no cross-reactivity, are rapid, and require little to no sample pretreatment or preconcentration. The main advantage of the array biosensor over existing technology is its ability to detect multiple analytes in multiple samples simultaneously on a single slide (19, 26). Multianalyte sensors increasingly appear in the literature, but most have yet to look at matrices more complex than buffer (30, 31). The ability to carry out multianalyte detection in complex samples is a clear advantage for screening food, water, or air samples for hazards either naturally occurring or deliberately introduced.

This work was supported by funding from the U.S. Food and Drug Administration.

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