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Recovery and detection of botulinum neurotoxins from a nonporous surface

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Abstract

We describe the adaptation of a sample recovery method for botulinum neurotoxins from stainless steel. Botulinum toxin was recovered from surfaces left to dry for up to 16 h and detected by either ELISA or EndoPep mass spectrometry methods. In addition, we demonstrate that this method can be used to evaluate the efficacy of surface decontamination procedures.

Keywords

Environmental microbiology; Sampling; Decontamination; ELISA; Endopep-MS

Botulinum neurotoxins (BoNT), of which there are 7 serotypes (A–G), are extremely toxic; the estimated adult human oral lethal dose, based on animal studies, is ≤70 μg (Arnon et al., 2001). Contamination of surfaces may occur as a result of intentional dissemination of BoNT or from laboratory accidents in pharmaceutical and biomedical research facilities. Currently, no published method for sampling and detecting BoNT from surfaces exists to assist responders to these incidents. The goal of this study was to develop a method to recover and detect BoNT from a nonporous surface.

We adapted a sampling method previously developed for the recovery of *Bacillus anthracis* from nonporous surfaces (Rose et al., 2004). BoNT recovered with Macrofoam swabs (Puritan Medical Products, Guilford, Maine) were detected as previously described using the Botulinum Toxin ELISA (Maslanka et al., 2011; Raphael et al., 2012). Select samples were also tested by Endopep mass spectrometry (Endopep-MS) (Wang et al., 2013).

Specific levels of BoNT in a total volume of 10 μl were evenly applied to a 4″×4″ stainless steel coupon and left to dry for either 1 or 16 h (overnight). Purified dichain (150 kDa) BoNT and complex toxins (BoNT and associated non-toxic proteins) were obtained from

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Metabiologics (Madison, WI). Culture supernatants were prepared using a *Clostridium botulinum* strain incubated anaerobically for 5 days. A Macrofoam swab, pre-moistened with ELISA kit dilution buffer, was brushed in both vertical and horizontal overlapping strokes over the entire surface of the coupon. The swab was then placed into a 15 ml conical tube containing 1 ml of the dilution buffer (obtained from the Botulinum Toxin ELISA kit) and vortexed for 1 min. Samples (100 μl) were loaded into duplicate ELISA wells. As previously described, the average background subtracted absorbance value (BSAV) for each sample was determined by subtracting the absorbance read at 690 nm from the absorbance read at 450 nm (Maslanka et al., 2011). A sample was considered positive when the BSAV of the sample was >0.2 above the BSAV of the kit dilution buffer (negative control).

Complex BoNT/A was added to coupons at a level of 1 ng, 100 pg or 10 pg. Toxin recovered from coupons spiked with 1 ng BoNT/A was detected after 1 or 16 h; however samples from coupons spiked at 100 pg and dried for 16 h were not positive by ELISA (Fig. 1). All samples from coupons spiked with either 1 ng or 100 pg, including those dried for 16 h, were positive by Endopep MS demonstrating that the toxin retained its enzymatic function even after drying (data not shown).

Based on the detection levels observed with BoNT/A, complex BoNT of serotypes A, B, E, and F were applied to the coupons at 500 pg and 1 ng levels, allowed to dry for either 1 or 16 h, and tested by ELISA. Type A and E toxins were detectable in all samples recovered from coupons spiked at 500 pg regardless of drying time (Fig. 2A). However, the BSAVs of the samples recovered from coupons spiked with 500 pg of serotype B or F toxins were too low to make any conclusions about the effect of drying time, as this level of toxin is close to the assay's detection limit for these serotypes. When spiked at the 1 ng level, toxin serotypes A, B, E, and F were detectable in the recovered samples; however, the serotype F toxin was not consistently detected at the 16 h drying time (Fig. 2B).

The efficacy of a surface decontamination procedure was evaluated by spiking stainless steel coupons with commercially prepared complex BoNT/A (1 ng), dichain BoNT/A (1 ng), or culture supernatant from a serotype A producing culture. After drying (1 h), spiked coupons were decontaminated by treatment with 10% freshly prepared bleach (containing 6.15% sodium hypochlorite), allowed to incubate at room temperature for 10 min, and wiped with gauze. Separate coupons spiked with identical toxin preparations were also prepared but not decontaminated. As shown in Fig. 3, the decontamination procedure effectively eliminated toxin from the non-porous surface regardless of the toxin preparation used. In order to ensure that the bleach treatment did not interfere with the ELISA, some control coupons were spiked with dilution buffer alone and subjected to decontamination or left untreated. Samples recovered from these control coupons were subsequently spiked with 1 ng of dichain BoNT/A. Any residual bleach following the decontamination procedure did not interfere with the ELISA since the BSAVs of the spiked control samples recovered from decontaminated coupons were equivalent to the BSAVs of the spiked control coupons that were not decontaminated.

The use of Macrofoam swabs followed by recovery in Botulinum Toxin ELISA kit dilution buffer and detection with the Botulinum Toxin ELISA is an effective method of examining

nonporous surface for contamination with BoNT. The method detected type A and E complex toxins at a level ≥500 pg and type B and F complex toxins at a level ≥1 ng. The Endopep MS method detected lower levels of complex BoNT/A than the Botulinum Toxin ELISA and further work is needed to compare the performance of these two in vitro methods for detection of toxin in recovered samples. Since Endopep MS detected enzymatic activity of toxin contaminating non-porous surfaces, such surfaces may still present a risk to individuals even up to 16 h following an intentional release or a laboratory accident. Finally, the method described in this work may be useful in monitoring the effectiveness of surface decontamination procedures.

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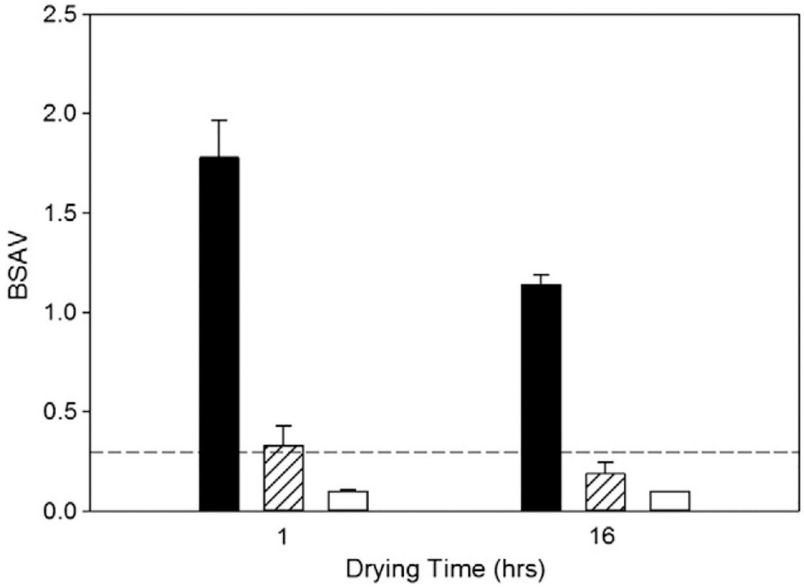

Fig. 1.

Recovery and detection of BoNT/A complex. Stainless steel coupons were spiked with 1 ng (black bars), 100 pg (striped bars), or 10 pg (white bars) and allowed to dry for either 1 h or 16 h. The Botulinum Toxin ELISA background subtracted absorbance value (BSAV) of triplicate recovered samples is shown. Error bars represent standard deviation. The threshold BSAV for a positive sample is indicated by the dotted line.

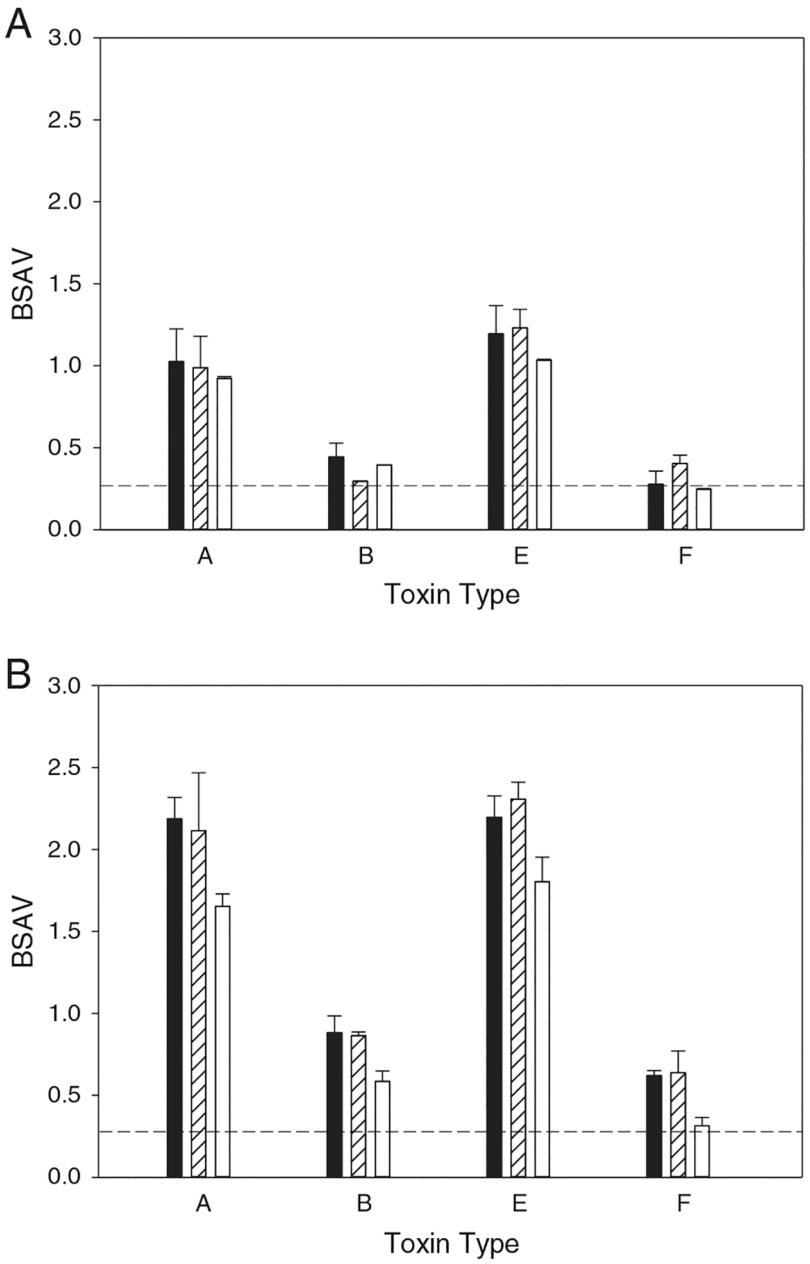

Fig. 2.

Recovery and detection of BoNT/A, BoNT/B, BoNT/E, and BoNT/F. Stainless steel coupons were spiked with the indicated complex toxins at a level of 500 pg (panel A) or 1 ng (panel B). Toxins were allowed to dry for either 1 h (striped bars) or 16 h (white bars). As a control, complex toxin was spiked directly in dilution buffer (black bars). The Botulinum Toxin ELISA background subtracted absorbance value (BSAV) of triplicate recovered samples is shown. Error bars represent standard deviation. The threshold BSAV for a positive sample is indicated by the dotted line.

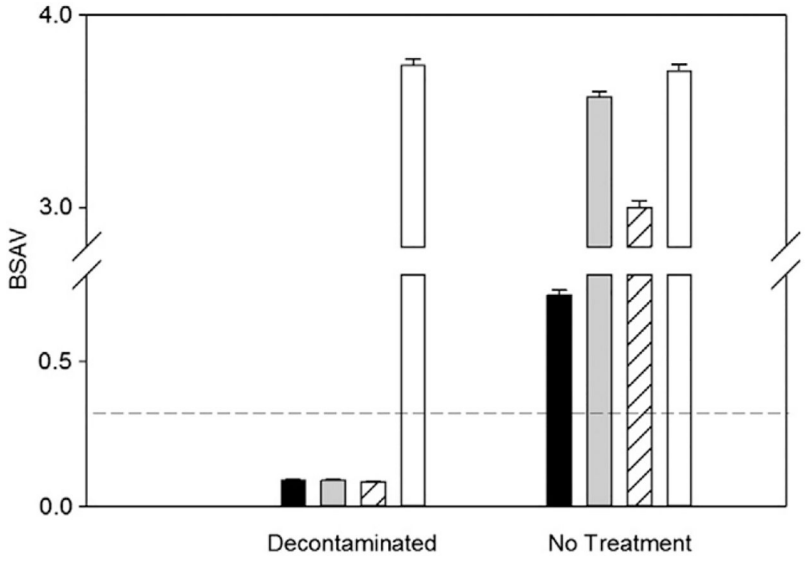

Fig. 3.

Recovery and detection of BoNT/A from decontaminated surfaces. Stainless steel coupons were spiked with 1 ng complex BoNT/A (black bars), 1 ng purified dichain BoNT/A (shaded bars), or 10 μl BoNT/A producing culture supernatant (striped bars) and allowed to dry for 1 h. Control coupons were spiked with dilution buffer alone. Purified dichain BoNT/A (1 ng) was added to samples recovered from the control coupons (white bars). Coupons were either treated with 10% bleach for 10 min (decontaminated) or left untreated (no treatment). The Botulinum Toxin ELISA background subtracted absorbance value (BSAV) of triplicate recovered samples is shown. Error bars represent standard deviation. The threshold BSAV for a positive sample is indicated by the dotted line.