SIMULTANEOUS DETECTION OF ABRIN, BOTULINUM TOXIN, RICIN, AND STAPHYLOCOCCUS ENTEROTOXIN B (SEB) IN BEVERAGES USING LUMINEX MULTIPLEX TECHNOLOGY

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ABSTRACT

Background: Numerous methods have been developed for the detection of proteinaceous toxins. However, antigen specific assays for the screening of samples containing an unidentified toxin are labor intensive, time consuming, and expensive. Alternatively, Luminex xMAP technology enabled the screening of a single sample for the presence of multiple antigens simultaneously.

Methods: Samples of beverages were spiked with varying concentrations of abrin, botulinum toxin A, ricin and Staphylococcus enterotoxin B (SEB) and analyzed using a cocktail of capture antibodies conjugated to spectrally unique coded beads. Multiple capture antibodies against different epitopes on the same antigen provided confirmatory tests within the same analysis and reduced the probability of false positives and false negatives that may occur when an epitope was inaccessible or altered. The assay also included two unique internal positive controls for antigen-antibody binding and quantitation. Several chemical and fluorimetric controls were also included such that a typical assay consisted of 17 different antibody-bead conjugates (17-plex).

Results: Abrin, botulinum toxin A, ricin, and SEB were detected in beverages at <5 ppb in the analytical sample; orders of magnitude less than would be considered a health concern. Variations in assay configuration demonstrated that maximum sensitivity was obtained using a monoclonal capture antibody and polyclonal detector antibodies.

Conclusions: Luminex technology provided an effective laboratory-based method for the simultaneous screening of beverages for abrin, botulinum toxin A, ricin and SEB.

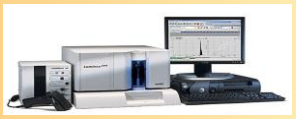
MATERIALS & METHODS

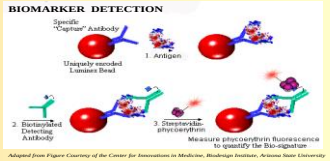
Ricin, Ricin A Chain, Ricin B Chain, and the agglutinin from castor beans (RCA-120) were obtained from Vector Labs. SEB was purchased from Sigma Chem. Co.. Botulinum toxins were purchased from either METAbiologics, Inc., List Biological Labs., Inc. or Wako chemicals. Abrin was prepared under contract for the FDA.

Sample Preparation & Analyses Beverage samples (100µL) spiked with varying amounts of the select agents were diluted first with 300µL PBST (PBS supplemented with 0.1% Tween-20) containing the internal calibration (dilution & detection) controls SC01 & SC02 at 3.3µg/mL and 13.3ng/mL, respectively. The samples were further diluted with 1.6mL 200 mM sodium phosphate, pH 6.8 (NaPi) and 2mL PBSTM (PBST supplemented with 5% w/v non-fat dried milk).

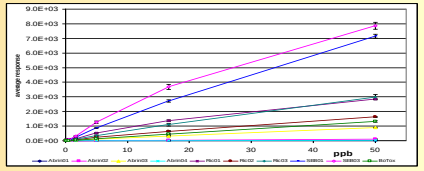
Typical analyses consisted of between 17 and 24 different antibody/reagent labeled bead sets. Multiple antibodies against a particular antigen provided a built-in confirmation of results. Fluorescence control (FC), negative control (NC), antibody control (AC), instrument control (IC), dilution controls (SC01 & SC02), and calibration controls (SC01 & SC02) facilitated monitoring of the operation of the instrument and the analytical procedure.

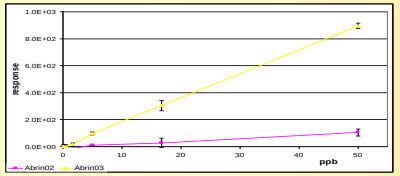
Instrumentation The assays were ran on either a Luminex 100 or a Bio-Plex Array Reader with Bio-Plex Manager 3.0 software.

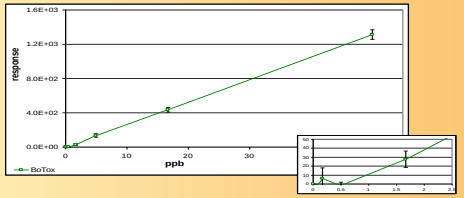


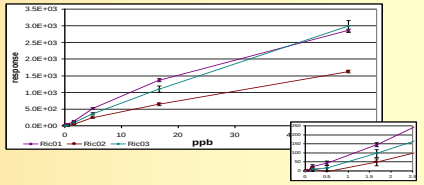


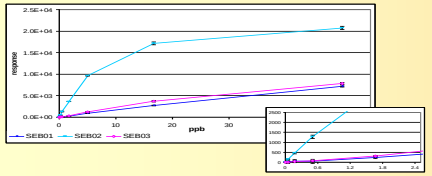
Adapted from Figure Courtesy of the Center for Innovations in Medicine, Biodesign Institute, Arizona State University

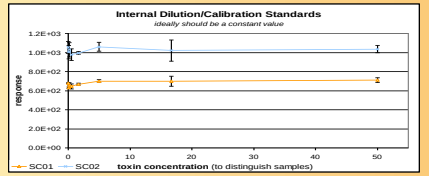




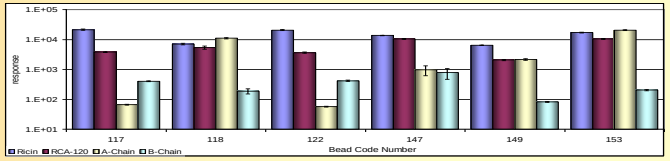








Analysis of Vegetable Juice spiked with a mixture of Abrin, Botulinum Complex (A), Ricin, and SEB. Beverages were initially spiked with a mixture of each toxin to a level of 0, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0, or 3 ppm. Sample preparation and analysis entailed a subsequent 60-fold dilution (40-fold dilution to prepare the sample followed by mixing 100 µL with 50 µL of beads) to yield the analytical sample with final concentrations of each toxin at 0, 0.05, 0.16, 0.5, 1.7, 5, 17, or 50 ppb. The figures above depict the responses measured versus the final concentration of each toxin in the analytical sample. *Similar results were obtained with fruit juice, dairy beverages, chocolate drink, and soda.*



Ricin Antibody Specificity Bead 117 contained Goat Polyclonal capture antibodies; 118 contained Monoclonal T271003-01 antibodies; 122 contained Monoclonal T141201-01 antibodies; 147 contained Monoclonal 58F4-2B7; 149 contained Rabbit Polyclonal; and 153 contained a mixture of Monoclonal antibodies T271003-01 & T141201-01. The concentration of antigen in the analytical sample was 166 ppb (10 ppm prior to dilution associated with preparation and analysis), similar results were obtained at 42, 10, and 2.6 ppb. *Note, the logarithmic vertical axis.*

CONCLUSIONS

Luminex xMAP / Bio-Plex technology enabled the simultaneous detection of abrin, botulinum toxin, ricin and SEB in beverages at concentrations orders of magnitude less than that associated with health concerns.

The use of antibodies with different specificities and affinities provided an internal confirmation and characterization for presumptive positive samples without the need to run multiple analyses.

Limits of Detection for the various agents in beverages in the analytical samples ranged from 6.7 - 0.05 ppb, with most between 2 - 0.5 ppb, depending on the beverage and particular antibody. As such, the limits of detection were comparable to ELISA tests but offered the added advantages of simultaneously detecting multiple toxins and by using multiple antibodies against any particular toxin provided built-in confirmation and antigen characterization.

The use of Internal Dilution/Calibration Standards (SC01 and SC02) facilitated detection and correction of errors in sample preparation (dilution) and operation of the instrument, thereby facilitating quantitative analyses.

ACKNOWLEDGEMENTS

Gratitude is expressed to Colin Wolcott (Tetracore, Inc.), Palmer Orlandi, Ph.D. (FDA), Robert Bull, Ph.D. (BDRD, USN, DoD), Peter Emanuel, Ph.D. (JPEO, Chem. Bio. Def., DoD), George Ziobro, Ph.D. (FDA), Benjamin Canas (FDA), Michael A. McLaughlin, Ph.D. (FDA), Mitchell J. Smith, Ph.D. (FDA), Douglas L. Park, Ph.D. (FDA), and Gregory Orlowski (JIFSAN, UMD), and Lynn L.B. Rust, Ph.D. (NIH) for contributions in substance or by helping to brainstorm areas of science, and creating an environment conducive to the work.

FDA

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