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technical BRIEF

Detecting Biological Contaminants in Water, Using Immunoassay Technologies

Seven immunoassay technologies evaluated for determining the presence of biotoxins in water

In the past, people in the United States have largely taken for granted the convenience of potable municipal water. However, the threat of intentional contamination of our water supplies is becoming a concern because of a rise in the number of terrorist acts around the world. As a result, there is much interest in technologies that can be used to detect a contamination event, as well as dispel or confirm the credibility of a threat. Such technologies include immunoassay tests that can be used to determine the presence of biotoxins and pathogens in water. The immunoassay devices are based on immunological interactions during which specific antibodies react with contaminants, or antigens, to produce a response indicating the presence of the contaminant.

U.S. EPA's Homeland Security Research Program (HSRP) develops products based on scientific research and technology evaluations. Our products and expertise are widely used in preventing, preparing for, and recovering from public health and environmental emergencies that arise from terrorist attacks. Our research and products address biological, radiological, or chemical contaminants that could affect indoor areas, outdoor areas, or water infrastructure. HSRP provides these products, technical assistance, and expertise to support EPA's roles and responsibilities under the National Response Framework, statutory requirements, and Homeland Security Presidential Directives.

Between 2004 and 2006, EPA evaluated seven immunoassay technologies:

- BADD™ Test Strips (ADVNT Biotechnologies)
- BioVerify Test Kits (BioVeris)
- EzyBot® A and EzyBot® B Test Kits (Pharmaleads)
- RAMP® Test Cartridges (Response Biomedical Corp.)
- BioThreat Alert® Test Strips (Tetracore, Inc.)
- Enzyme Linked Immunosorbent Assay (Tetracore, Inc.)
- QTL Biosensor (QTL Biosystems LLC)

EPA tested each immunoassay technology's ability to detect specific biotoxins, as well as its propensity to register false positive and false negative responses as a result of interfering compounds, cross-reactive species, or matrix-specific information. Because immunoassay technologies are expected to serve mainly as screening tools in water monitoring scenarios, this testing produces only qualitative results (i.e., results indicate only the presence or absence of a contaminant, not a concentration level). Each of the seven technologies was evaluated for:

- Contaminant presence/absence (i.e., accuracy of the technology)
- False positive/false negative response
- Consistency
- Lowest detectable concentration
- Other performance factors

Test Design

Table 1 identifies the immunoassay technologies tested using various water types fortified (spiked) separately with contaminants, interfering compounds, and cross-reactive species (i.e., a compound or spore that is chemically similar to a contaminant of interest).

Table 1. Immunoassay Technologies, Contaminants, Cross-Reactive Species, Interfering Compounds

Technologies	Contaminants	Cross-Reactive Species	Interfering Compounds
BADD™ Test Strips	Anthrax Botulinum toxins Ricin	*B. thuringiensis* Lipopolysaccharide Lectin	Calcium Magnesium Humic Acid Fulvic Acid
BioVerify Test Kits	Botulinum toxin A Ricin	Lipopolysaccharide Lectin	
EzyBot® A and EzyBot® B Test Kits	Botulinum toxins A and B	Lipopolysaccharide	
RAMP® Test Cartridges	Anthrax Botulinum toxins Ricin	*B. thuringiensis* Lipopolysaccharide Lectin	
BioThreat Alert® Test Strips	Anthrax Botulinum toxins Ricin	*B. thuringiensis* Lipopolysaccharide Lectin	
Enzyme Linked Immunosorbent Assay	Anthrax Botulinum toxins Ricin	*B. thuringiensis* Lipopolysaccharide Lectin	
QTL Biosensor	Anthrax Ricin	*B. thuringiensis* Lectin	

Three types of water samples were tested in these evaluations: performance test (PT), drinking water (DW), and quality control (QC). PT samples were prepared with deionized (DI) water and fortified with the target contaminant, an interferent, both, or only a cross-reactive species. Contaminant-only PT samples were tested in a series of concentrations that included the accepted lethal/infective dose, the vendor-stated detection limit, and approximately 5, 10, and 50 times the identified detection limit.

DW samples were tested to determine the effects of matrix-specific characteristics (e.g., location, filtering) on the technology being evaluated. DW samples were collected from four geographically diverse municipal sources that varied in source (ground water or surface water), treatment (filtered or unfiltered), and disinfection process (chlorination or chloramination). In order to evaluate the effect of a concentrated DW sample, 100 L of DW was dechlorinated and then concentrated to 250 mL, using an ultrafiltration sample concentration method. Each DW sample (nonconcentrated and concentrated) was analyzed without adding any contaminant, as well as after fortification with individual contaminants at concentration levels approximately 10 times greater than the immunoassay test kit detection limit. Interferent compounds, cross-reactive species, and DW were used to determine the immunoassay's propensity to register false positive and false negative responses.

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All PT and DW samples were analyzed in triplicate when possible. Fewer replicates were analyzed if vendor-supplied materials were limited. The results of each replicate sample set were reported as a ratio of the number of positive results to the total number of replicates (e.g., 0/3, 1/3). Method blank QC samples consisted of at least 10% of all samples.

Performance and Results

The accuracy of the technology was determined by dividing the number of positive responses by the overall number of analyses of spiked contaminant-only PT samples. A false positive response was defined as a positive response from DW samples that were either spiked with a potential interferent or cross-reactive compound, or not spiked at all. A false negative response was defined as a negative response from any sample that was spiked with a contaminant concentration greater than the lowest detectable concentration.

Consistency or reproducibility of results was determined by calculating the percentage of individual test samples that produced positive or negative responses without variation within replicates. The lowest detectable concentration for each contaminant was determined to be the concentration level at which at least two of the three replicates generated positive responses. Table 2 summarizes the results for each evaluation parameter and technology.

Table 2. Summary of Results

Technology	Contaminant	Contaminant Presence/ Absence	False Positive Responses	False Negative Responses	Consistency	Lowest Detectable Concentration
BADD™ Test Strips	Anthrax	14/24	0	1	90%	$4x10^7$ cfu/mL
	Botulinum toxin A	7/12	1	0	84%	5 mg/L
	Botulinum toxin B	2/21				ND
	Ricin	9/21	0	0	100%	20 mg/L
BioVerify Test Kits	Botulinum toxin A	9/22	0	6	100%	0.0005 mg/L
	Ricin	15/22	0	3	97%	0.0005 mg/L
EzyBot® A and EzyBot® B Test Kits	Botulinum toxin A	19/22	0	9	100%	0.05 mg/L
	Botulinum toxin B	22/22	0	6	97%	0.01 mg/L
RAMP® Test Cartridges	Anthrax	8/20	0	0	96%	$8x10^8$ cfu/mL
	Botulinum toxin A	7/12	0	1	95%	2 mg/L
	Botulinum toxin B	0/18				ND
	Ricin	12/15	0	0	100%	5 mg/L
BioThreat Alert® Test Strips	Anthrax	12/19	2	6	96%	$8x10^7$ cfu/mL
	Botulinum toxin A	12/12	3	0	92%	0.01 mg/L
	Botulinum toxin B	13/15				0.05 mg/L
	Ricin	15/15	2	1	100%	0.035 mg/L
Enzyme Linked Immunosorbent Assay	Anthrax	15/36	2	0	100%	$8x10^6$ cfu/mL
	Botulinum toxin A	9/12	0	5	98%	0.02 mg/L
	Botulinum toxin B	7/15				ND
	Ricin	12/15	0	0	100%	0.0075 mg/L
QTL Biosensor	Anthrax	10/15	22	3	72%	$5x10^5$ cfu/mL
	Ricin	12/15	2	2	90%	0.25 mg/L

The most accurate results were obtained in three instances, using two separate technologies: the EzyBot® B test kit accurately detected the presence of the botulinum toxin B in 22/22 tests and the Bio-Threat® Alert test strips detected 12/12 and 15/15 for botulinum toxin A and ricin, respectively.

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The two least accurate were 0/18 and 2/21, both botulinum toxin B results from the RAMP® Test Cartridges and the BADD™, respectively. Review of the associated QA plan identified that the vendors did not indicate whether or not their technology was specific to a particular type (A or B) of botulinum toxin. The results suggested that at least two of the technologies were designed for botulinum toxin A, and this was confirmed by the vendors.

The maximum number of false positives for anthrax tests was 22 out of 22, using the QTL Biosensor, with the remaining tests exhibiting 3 or fewer false positives. The maximum number of false negatives was 9 for the botulinum toxin A tests, using the EzyBot® A Test Kit. Thirteen of the 18 biotoxin tests achieved 95% consistency or above, while the minimum consistency was 72%. The detection limits for each immunoassay technology are also indicated in the table for the respective contaminants.

CONTACT INFORMATION

For more information, visit the EPA Web site at www.epa.gov/nhsrc.

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