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Performance Assessment of Three Commercial Assays for Direct Detection of *Bacillus anthracis* Spores

Bacillus anthracis, the cause of anthrax, has been used as a bioterrorism agent. Because the isolation and identification of *B. anthracis* by culture can take days, first response units (hazardous materials [HAZMAT], firemen, police, and hospital personnel) desire a quick and easy test that can be done in the field to detect possible *B. anthracis* contamination (1, 4). To our knowledge, there are no peer-reviewed published data on commercially available kits that could guide first responders in their search for such rapid detection methods. We tested three lateral flow immunoassay kits that are designed to test for *B. anthracis* at 10^4 to 10^5 spores per sample: (i) Anthrax BioThreat Alert (BTA) test strips (Tetracore, Gaithersburg, Md.), (ii) BioWarfare Agent Detection Devices (BADD) (Osborne Scientific, Lakeside, Ariz.), and (iii) Anthrax (spore) SMART II (New Horizons Diagnostics, Columbia, Md.). These tests require little technician time and training, and results are available within 15 min.

This study was conducted at the Florida Department of Health Laboratory in Tampa, Fla., and employed *B. anthracis* Pasteur (CDC BC 3132) and *Bacillus cereus* and *Bacillus thuringiensis* from our culture collection. Spores were added to the buffer provided by the manufacturer to achieve 10^2 to 10^6 (*B. anthracis*) or 10^6 (*B. cereus* and *B. thuringiensis*) spores per sample. The range of 10^2 to 10^5 for *B. anthracis* spores was chosen in order to include the manufacturer's claims of sensitivity. We also tested 10^6 spores in order to achieve a clear, easy-to-read positive result. Because one of the kits did not consistently detect spores at the upper limit (10^5), we tested 10^6 spores more than once to see if detection was consistent. All tests were performed according to the manufacturer's instructions and were allowed to proceed for 15 min, although the positive results were recognized within 5 min. Spore concentrations were verified by viable plate counts on Trypticase soy agar (Remel, Lenexa, Kans.) in duplicate.

All of the assay kits were able to detect *B. anthracis* at 10^6 spores (Table 1). While BADD and SMART II consistently detected 10^5 spores, Anthrax BTA detected 10^5 spores only once in eight attempts (12.5%). BADD yielded a sensitivity of 35.7%, SMART II demonstrated a sensitivity of 41.6%, and Anthrax BTA had a sensitivity of 30.43% (Table 1). Both BADD and Anthrax BTA had a specificity of 100%, while SMART II showed a positive reaction with *B. thuringiensis*, giving a specificity of 75%.

First responders desire a method that accurately detects <100 *B. anthracis* spores (4). When areas contain numbers of spores lower than the test assay detection limit, the resulting false-negative findings may lead first responders to employ relaxed safety precautions. Although the assays tested are self-contained and easy to use in the field, their sensitivity fails to meet that of our laboratory experiments (e.g., <10 spores on environmental swab samples) (2).

The costs per assay of using the Anthrax BTA, BADD, and SMART II assays are $19.80, $42.50, and $52.10, respectively. These high costs may preclude their being used during times of high sample volume, as was seen during the fall of 2001 when the Laboratory Response Network tested >84,000 samples (3). Only three kits and a limited number of assays were performed due to the unavailability of other products and the cost. Tetracore sells a test strip reader (Guardian Bio Threat Alert Test Strip Reader) that costs approximately $4,000. However, this instrument may only increase the detection level by 1 to 2 logs according to the manufacturer (Guardian Bio-Threat Alert System technical information, Alexeter Technologies, Wheeling, Ill.). When the sensitivity is increased and the costs are reduced, these assay kits may hold promise for field detection by first responders.

TABLE 1. Sensitivity and specificity of three assay kits for *B. anthracis*, *B. cereus*, and *B. thuringiensis*[a]

Assay	*B. anthracis*													*B. cereus* (10^6 spores)		*B. thuringiensis* (10^6 spores)		% Overall specificity[b]
	10^6 spores		10^5 spores		10^4 spores		10^3 spores		10^2 spores		Total		% Overall sensitivity					
	No. + (*n*)	%	No. + (*n*)	%	No. + (*n*)	%	No. + (*n*)	%	No. + (*n*)	%	No. + (*n*)	%		No. + (*n*)	%	No. + (*n*)	%	
Anthrax BTA	6 (6)	100	1 (8)	12.5	0 (5)	0	0 (2)	0	0 (2)	0	7 (23)	30.43	30.43	0 (2)	0	0 (2)	0	100
BADD	2 (2)	100	3 (3)	100	0 (3)	0	0 (2)	0	0 (4)	0	5 (14)	35.71	35.71	0 (2)	0	0 (2)	0	100
SMART II	2 (2)	100	3 (3)	100	0 (3)	0	0 (2)	0	0 (2)	0	5 (12)	41.67	41.67	0 (2)	0	1 (2)	50	75

[a] The results show the number of positive tests (No. +) out of *n* attempts. Note that the manufacturers state the kits can detect as few as 10^4 spores per sample.
[b] For all spore concentrations of all organisms.

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Debra King
Vicki Luna*
Andrew Cannons
Jacqueline Cattani
USF Center for Biological Defense
College of Public Health
University of South Florida
Tampa, FL 33612

Phil Amuso
Bureau of Laboratories
Florida Department of Health
Tampa, FL 33612

*Phone: (813) 974-3873
Fax: (813) 974-1479
E-mail: vluna@bt.usf.edu