Journal of Hazardous Materials 172 (2009) 559–565

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

An evaluation of suspicious powder screening tools for first responders

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ARTICLE INFO

Article history:
Received 7 January 2009
Received in revised form 28 May 2009
Accepted 29 May 2009
Available online 9 June 2009

Keywords:
Detection
Suspicious powders
Bio-threat agents
Biological weapons
Field screening

ABSTRACT

Field screening tools are required which would allow first responders to quickly ascertain if a suspicious powder poses a potential threat necessitating additional testing for biological pathogens such as *Bacillus anthracis*. In this study, three commercially available generic screening technologies were evaluated for the effectiveness to accurately differentiate between a hoax powder and a true biological threat. The BioCheck® Kit was able to detect the following biological agents 1×10^8 CFU of *B. anthracis* Sterne (washed 4 times), 1×10^7 CFU of *B. anthracis* ΔSterne (washed 2 times), 1×10^7 CFU of *Yersinia pestis* A1122, and 100 μg of ricin. The Prime Alert™ kit was able to detect 2×10^{10} CFU of *B. anthracis* ΔSterne 4×, 1×10^9 CFU of *B. anthracis* ΔSterne 2×, and 1×10^8 CFU of *Y. pestis* A1122. The Prime Alert™ kit was not able to detect ricin. The Profile®-1 kit was able to detect 1×10^4 CFU of *B. anthracis* ΔSterne 4× and *B. anthracis* ΔSterne 2×, and 1×10^6 CFU of *Y. pestis* A1122. The Profile®-1 kit was not able to detect ricin. All of the kits showed positive results for powders containing components specifically targeted by the particular technology being used. Each technology assessed in this evaluation employs a different mechanism for the detection of biological materials and it is important that first responders are aware of the strengths and the limitations of each system so that they can effectively employ the technology to protect the homeland.

1. Introduction

Seven years after letters laced with *Bacillus anthracis* spores killed seven people across the nation first responders are still dealing with numerous suspicious powder incidents each month. These incidents disrupt the community and cost taxpayers thousands of dollars. Mimi Hall of USA Today reported on October 16, 2008 that in the past year the U.S. Postal Inspection Service has responded to 2893 incidents involving suspicious powders and the FBI investigated more than 900 biological incidents. It is widely recognized that what is needed is a rapid and reliable method to rule out hoax powder incidents in order to quickly restore commerce and ease safety concerns. Towards this end a panel of experts proposed an economical 5-step pre-screening kit to rule out suspected biological threats in a powdered form without the need to engage costly test kits such as PCR or hand-held assays [1]. The 5-step method relied on measuring specific properties of a suspicious powder including apparent particle size, solubility in water, acidity, and protein content to determine whether a powder had the potential to be of biological origin. One aspect that made the proposed 5-step method attractive was that it suggested the use of commercially available urine test strips which dropped the test kit cost to less than two dollars making it affordable and easy to employ in the field [1]. In 2004 the Department of Defense and Department of Homeland Security conducted a study to determine if the 5-step method was effective for field use. The study found that, although the 5-step method could be useful in discriminating hoax powders from true threats, the risk of false negatives using the method was a concern and the method was not recommended to responders [2,3]. Recently several commercial systems have been introduced which seek to fill the gap by providing cost effective biological screening tools that can non-specifically determine the presence of a biological material in a suspicious powder. The DoD's Edgewood Chemical Biological Center evaluated three such systems to determine if they were effective for use by first responders. The key attributes of the three systems are summarized in Table 1.

The BioCheck® Powder Screening Test Kit (20/20 GeneSystems, Rockville, MD) is a swab based test kit which utilizes protein detection and pH testing technology for screening powders [4]. The Prime Alert™ system (GenPrime, Spokane, WA) employs a DNA-based fluorescent detection technology to determine the presence of bacteria or viruses [5,6]. A penetrating dye is used that only fluoresces when directly bound to nucleic acid material and a battery operated reader measures the result. The Prime Alert™ system is packaged with antibody-based immunoassay test strips specific for ricin and botulinum toxins, but these test strips were not assessed in this study. Finally the Profile®-1 System (New Horizons Diagnostics, Columbia, MD) utilizes technology that detects adenosine

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0304-3894/$ – see front matter
doi:10.1016/j.jhazmat.2009.05.142

Table 1
General kit overview.

Kit	Manufacturer	Cost/test	Total time	Ancillary equipment required for testing at an additional cost	Technology target
BioCheck®	20/20 GeneSystems	$22.80	5 min	No	Protein
Prime Alert™	GenPrime	$150.00	5 min	Yes	DNA
Profile®-1	New Horizon Diagnostics	$4.00	20 min	Yes	ATP

triphosphate (ATP), a component produced by all living cells [7]. A luciferin–luciferase (LL) reaction occurs in the presence of ATP which is measured by a microluminometer [8]. This technology is coupled with antibody-based immunoassay toxin test strips and a sample collection kit that works to prevent clogging of a filter used in the system.

All of the kits mentioned above are currently being used by first responders and were tested with the biological agents *B. anthracis*, *Yersinia pestis*, and ricin toxin.

2. Materials and methods

2.1. Bacterial strains and reagent preparation

Lemco Sporulation Plate Method. *B. anthracis* ΔSterne (4×) (*B. anthracis* ΔSterne 4×)—*B. anthracis* ΔSterne UCC# BAC1056 (Critical Reagents Program, Edgewood, MD) was streaked for isolation on tryptic soy agar (TSA, Becton Dickenson, Sparks, MD). Following an overnight incubation at 37 °C a single colony was inoculated into a flask containing 10 mL sterile tryptic soy broth (TSB, Becton Dickenson, Sparks, MD) and incubated at 37 °C for 24 h at 200 rpm. Four hundred microliters of the overnight culture were spread onto Lemco sporulation agar plates and incubated at 37 °C for 7 days until greater than 95% sporulation was observed. Sporulation plates were then placed at 4 °C for 2 h prior to processing. Each plate was washed twice with 10 mL sterile cold diH_2O to remove growth from agar surface and transferred to 450 mL centrifuge bottles. Centrifugation was done at 8500 rpm for 15 min at 4 °C. Spore pellets were washed 3 times with 100 mL ice-cold sterile distilled H_2O, and once with 100 mL of 70% ethanol (Acros, Morris Plains, NJ) while decanting the supernatant after each centrifugation. An additional heat shock at 65 °C for 30 min was performed prior to the final wash. The final pellets were resuspended in 100 mL ice-cold sterile diH_2O and combined into one 2 L glass bottle. One to ten serial dilutions of the final stock culture were made in sterile diH_2O, plated on TSA, and incubated at 37 °C for 24 h. Colonies were counted and stored electronically by Q-count (Spiral Biotech, Norwood, MA) and the final spore concentration calculated (CFU/mL).

Fermentation Method. *B. anthracis* ΔSterne (2×) (*B. anthracis* ΔSterne 2×)—*B. anthracis* ΔSterne UCC# BAC1056 (Critical Reagents Program, Edgewood, MD) was streaked for isolation on tryptic soy agar (TSA, Becton Dickenson, Sparks, MD). Following an overnight incubation a single colony was inoculated into a flask containing 10 mL sterile nutrient broth (NB, Becton Dickenson, Sparks, MD) and incubated at 37 °C for 24 h. The 10 mL starter culture was inoculated into 1700 mL of 2× NB expansion culture and incubated for 24 h at 37 °C. The expansion culture was then transferred to an 8.5 L fermenter containing 6120 mL G media with 680 mL of 10× trace minerals and allowed to sporulate for 96 h. After 96 h sporulation, 750 mL of growth media was transferred into each of 11 1 L centrifuge bottles and centrifuged at 8500 rpm for 30 min at 4 °C in a Sorvall SLC6000 rotor. Each pellet was then washed twice with 100 mL ice-cold sterile diH_2O and centrifuged at 8500 rpm for 15 min at 4 °C with the supernatant decanted after each wash. The final pellets were resuspended in 100 mL ice-cold diH_2O and combined into one 2 L glass bottle. One to ten serial dilutions of the final stock culture were made, plated on TSA, and incubated at 37 °C for 24 h. Colonies were counted and stored electronically by Q-count (Spiral Biotech, Norwood, MA) and final spore concentration was calculated (CFU/mL).

Y. pestis A1122 (UCC# YERS078, LOT# WD060607A) (*Y. pestis* A1122) frozen cell culture (Critical Reagents Program, Edgewood, MD) was acquired and streaked for isolation on tryptic soy agar (TSA, Becton Dickenson, Sparks, MD). A single colony was inoculated into a flask containing 10 mL Brain Heart Infusion Broth (BHI, Becton Dickenson, Sparks, MD) and incubated at 30 °C for 48 h at 200 rpm. The 20 mL of starter culture was then transferred to 500 mL BHI and returned to 30 °C incubator for 24 h at 200 rpm. 500 mL growth culture was transferred to 450 mL centrifuge bottles and then centrifuged at 3000 rpm for 12 min at 4 °C. The cell pellet was then washed twice with ice-cold, sterile 1× Phosphate Buffer Saline (PBS, EMD Chemicals Inc., Gibbstown, NJ) and centrifuged between washes. The final pellet was resuspended in 50 mL ice-cold, sterile 1× PBS and stored at 4 °C. One to ten serial dilutions of the final stock culture were made in sterile 1× PBS, plated on TSA, and incubated at 30 °C for 48 h. Colonies were counted and stored electronically by Q-count (Spiral Biotech, Norwood, MA) and the final concentration was calculated (CFU/mL).

Ricin toxin (RCA_{60}) was purchased from the Vector Laboratories at a concentration of 5 mg/mL and stored at 4 °C. The panel of powders used in this study represents commonly encountered hoax powders likely to be sampled by first responders. The following powders were used in this study: *Bacillus thuriengensis* DiPel® powder, Carnation powdered milk, Cremora powdered coffee creamer, Johnson & Johnson baby talcum powder, Gold Medal all-purpose flour, Domino's powdered sugar, Fleischman's brewers yeast, Dry wall dust, Morton's iodized salt, Chalk dust, Gold Bond medicated foot powder, Kaolin, Bentonite, and 3 other coded powders.

2.2. BioCheck® Kit test methods

One milliliter of each dilution of *B. anthracis* and *Y. pestis* was spun down in a microcentrifuge tube, resuspended in a minimal volume of solution from the protein detection tube and then added back to the protein detection tube. Manufacturer's recommendations stated that the volume in the tube should not be significantly altered and so only the pellets were used in the kit testing. The swab containing the detection reagents was added to the protein detection tube and allowed to incubate for five minutes at 25 °C. One milliliter of each dilution was spun down, resuspended in a minimal volume of solution from the pH tube, and then added back to the pH tube. The swab containing the detection reagents was added to the pH tube and allowed to incubate for five minutes at 25 °C. The color change for both the protein and pH tubes was recorded. For ricin toxin test 100 μl of each toxin dilution was added directly to the protein detection and pH tubes and then processed according to the manufacturer's instructions above. For testing with powders alone using the BioCheck® Kit, 10 mg of powder was added to both the protein detection and pH tubes and processed as described above. For testing of powders spiked with biological agents one milliliter of each dilution of *B. anthracis* and *Y. pestis* which was spun down in a microcentrifuge tube, resuspended in a minimal volume of solution and then added back to the protein or pH detection tubes. Ten milligrams of the suspicious powder was added to the tube and the swab containing the detection or pH reagents was then added and allowed to incubate for five min at 25 °C. The color change for both

the protein and pH tubes was then recorded. The following modification to the protocol was made to test for ricin toxin: 100 μL of a ricin dilution was added directly to the protein detection and pH tubes followed by the addition of 10 mg of powder to each tube. The manufacturer's instructions were then followed.

2.3. Prime Alert™ kit test methods

One milliliter of each dilution of *B. anthracis* and *Y. pestis* was spun down in a microcentrifuge tube, the supernatant was decanted and the resulting pellets were air-dried. The pellets were resuspended in buffer from the dropper bottle according to the manufacturer's instructions. Eight drops of Cell Prep Solution was dispensed into the supplied glass vial followed by the addition of the Reaction Solution to the glass vial. The sample was then added to the dropper bottle and mixed. Four drops of the mixed sample were dispensed from the dropper bottle into the glass vial. The vial was then inserted into the Prime Alert™ reader. The following modification to the protocol was made to test for ricin toxin: 100 μL of a ricin dilution was added directly to the dropper bottle and then processed according to the manufacturer's instructions above. For testing with powders alone 10 mg of powder was added to the dropper bottle and processed according to manufacturer's instructions. For testing of powders spiked with biological agents 1 mL of each dilution was spun down in a microcentrifuge tube, the cell pellets were air-dried, resuspended in buffer and then added back to the dropper bottle. Ten milligrams of powder was then added to the dropper bottle and the test was performed according to manufacturer's instructions.

2.4. Profile®-1 system test methods

(for *B. anthracis* and *Y. pestis*) This system necessitates measuring an unincubated sample as well as a sample that has been incubated in TSB. The ATP from an unincubated sample was first measured to determine the actual RLU (relative light units) indicative of the bacteria present in the sample. The incubated sample was then also measured for ATP reflected as incubated RLU. The difference between the incubated RLU and the actual RLU is a representative of spores present in the sample. For some samples, both the incubated RLU and the unincubated RLU values were extremely high, however, when the actual RLU value was calculated, it was negative even though both sample values were high (indicated in the tables). The manufacturer's instructions for determining the actual RLU are described in the following steps: 100 mL of the dilution was added to the Filtravette™ with the excess liquid being expressed using a Positive Pressure Device. Four drops of the SRA was added to the Filtravette™ and the liquid expressed. The additional four drops of the SRA were added to the Filtravette™ and the liquid expressed. The Filtravette™ was inserted into the luminometer followed by the addition of two drops of BRA. Fifty microliters of the L/L reagent was added and mixed well. The light units produced were then measured by the luminometer. The protocol for determining the incubated RLU contained an additional step of adding 150 μL of TSB and incubating the sample at 37 °C for 15 min which was added between the two SRA washes. The following modification to the protocol was made to test for ricin toxin: 10 μL of a ricin dilution was added directly to the Filtravette™ and then processed according to the manufacturer's instructions above. For testing with powders alone using the Profile®-1 System, 10 mg of powder was resuspended in 1 mL of H_2O. One hundred microliters of the powder solution was added to the Filtravette™ and processed according to manufacturer's instructions for both actual RLU and incubated RLU measurements. For testing of powders spiked with biological agents, 10 mg of powder was resuspended in 1 mL of H_2O. One hundred microliters of the powder solution was added to the Filtravette™. One hundred microliters of the agent dilution was also added to the Filtravette™ and the manufacturer's instructions were followed for actual RLU and incubated RLU measurements.

For all testing, readings obtained from each technology were then converted to a +/− rating system where +++ was equivalent to an extremely positive result, ++ was a positive result, + was a slightly positive result, while a − was equivalent to a negative result.

3. Results

3.1. Summary of the kit evaluation

In order to evaluate the effectiveness of the generic biological screening technologies, the limit of detection (LOD) was determined for each technology followed by testing with a panel of common hoax powders in order to show the kit's ability to differentiate hoax powders from biological agents. Mixtures of each agent and powder were also analyzed for the kit's ability to detect the agent in the presence of a potential interference matrix. Finally each technology in this study was compared to the specifications reported by the individual manufacturers. Laboratory testing was conducted by three independent evaluators and consisted of five types of samples:

(1) Pure agent—unmixed *B. anthracis*, *Y. pestis*, and ricin toxin.
(2) Fifteen powders from the DoD suspicious powders panel.
(3) Fifteen powders from the DoD suspicious powders panel spiked with one of the pure agents from above.
(4) Positive control which is expected to trigger a positive result.
(5) Negative control or blank sample which is expected to render a negative result.

For all testing, readings obtained from each technology were then converted to a +/− rating system where +++ was equivalent to an extremely positive result, ++ was a positive result, + was a slightly positive result, while a − was equivalent to a negative result.

3.2. Limit of detection testing

The LOD of agents, or amount of agent detected by the technology, was determined for all kits over a range of concentrations with every measurement made at least three times. A single preparation of *Y. pestis* and ricin was tested with each kit. Because previous studies have indicated that extensively washed spore preparations can be more difficult to detect two preparations of *B. anthracis* were produced. One was washed twice [*B. anthracis* spores (2×)] and under microscopic examination was found to have traces of cellular debris (data not shown). A second more extensively washed preparation [*B. anthracis* spores (4×)] showed no traces of residual cellular debris. The BioCheck® Kit could detect 1×10^8 CFU of *B. anthracis* spores (4×) (Table 2). The less extensively washed preparation was easier to detect for the BioCheck® Kit with an LOD of 1×10^7 CFU. The *Y. pestis* had a similar LOD as *B. anthracis* 2× of 1×10^7 CFU. Ricin toxin, a pure protein preparation, had an LOD of 100 μg. All of the samples had a pH close to neutral, indicated by the color produced in the pH tube. The Prime Alert™ kit was able to detect 2×10^{10} CFU of *B. anthracis* 4× (Table 2). The Prime Alert™ kit had a lower LOD for the *B. anthracis* 2× preparation, 1×10^9 CFU, which was washed only two times. *Y. pestis* was detected at an even lower amount, 1×10^8 CFU. As expected, ricin was not detected by this kit because the purified ricin preparation does not contain DNA, the constituent detected by this kit. The Profile®-1 kit produced LOD's of 1×10^4 CFU for both *B. anthracis* 4× and 2× (Table 2). The LOD for *Y. pestis* was 1×10^6 CFU. Ricin was not detected by

Table 2
Agent LOD testing.

Agent added	Amount added (CFU)	BioCheck®	Prime Alert™	Profile®-1
Ba 4× wash	1×10^9	+++	–	+++
	1×10^8	+	–	+++
	1×10^7	–	–	+++
	1×10^6	–	–	+++
	1×10^5	–	–	++
Ba 2× wash	1×10^9	+++	+	NT
	1×10^8	+++	–	Clogged filter
	1×10^7	+	–	+++
	1×10^6	–	–	+++
	1×10^5	–	–	+
Yp A1122	1×10^9	+++	+++	NT
	1×10^8	++	++	Clogged filter
	1×10^7	+	–	–[a]
	1×10^6	–	–	+
	1×10^5	–	–	–
Ricin toxin (RCA_{60})	500 μg	++	–	–
	100 μg	+	–	–
	10 μg	–	–	+
	1 μg	–	–	–

[a] Both incubated and unincubated samples were extremely positive, but the difference in the two samples was negative.

Table 3
Powder panel testing.

Powder	BioCheck®	Prime Alert™	Profile®-1
Yeast	+++	++	–[a]
DiPel®	+++	+++	+++
Dry milk	+++	–	–
Powder A	+++	–	–
Flour	+++	–	+
Coffee creamer	++	–	–
Salt	–	–	–
Powdered sugar	–	–	–
Talcum powder	–	–	+
Chalk dust	–	–	–
Foot powder	–	–	–
Powder B	–	–	–
Kaolin	–	–	–
Spackling powder	–	–	–
Powder C	–	–	–
Bentonite	–	–	Clogged filter

[a] Both incubated and unincubated samples were extremely positive, but the difference in the two samples was negative.

Table 4
BioCheck® agent and powder panel testing.

Powder	Agent concentration	Ba 4× wash	Ba 2× wash	Yp A1122	Agent concentration	Ricin toxin
		Protein	Protein	Protein		Protein
Yeast	1 × LOQ	+++	+++	+++	1 × LOQ	+++
	5 × LOQ	+++	+++	+++	3 × LOQ	+++
DiPel®	1 × LOQ	+++	+++	+++	1 × LOQ	+++
	5 × LOQ	+++	+++	+++	3 × LOQ	+++
Dry milk	1 × LOQ	+++	+++	+++	1 × LOQ	+++
	5 × LOQ	+++	+++	+++	3 × LOQ	+++
Powder A	1 × LOQ	+++	+++	+++	1 × LOQ	+++
	5 × LOQ	+++	+++	+++	3 × LOQ	+++
Flour	1 × LOQ	+++	+++	+++	1 × LOQ	+++
	5 × LOQ	+++	+++	+++	3 × LOQ	+++
Coffee creamer	1 × LOQ	+++	++	++	1 × LOQ	+++
	5 × LOQ	+++	++	++	3 × LOQ	+++
Salt	1 × LOQ	+	+	–	1 × LOQ	+
	5 × LOQ	+++	+++	++	3 × LOQ	+++
Powdered sugar	1 × LOQ	+	+	–	1 × LOQ	+
	5 × LOQ	+	+++	++	3 × LOQ	++
Talcum powder	1 × LOQ	+	+	–	1 × LOQ	+
	5 × LOQ	+++	++	+	3 × LOQ	++
Chalk dust	1 × LOQ	+	+	–	1 × LOQ	+
	5 × LOQ	+++	++	++	3 × LOQ	++
Foot powder	1 × LOQ	+	+	–	1 × LOQ	+
	5 × LOQ	+++	++	++	3 × LOQ	++
Powder B	1 × LOQ	+	+	–	1 × LOQ	–
	5 × LOQ	+++	+++	+	3 × LOQ	–
Kaolin	1 × LOQ	–	+	–	1 × LOQ	+
	5 × LOQ	+++	+	+	3 × LOQ	++
Spackling powder	1 × LOQ	+	–	–	1 × LOQ	+
	5 × LOQ	++	++	+	3 × LOQ	++
Powder C	1 × LOQ	–	–	–	1 × LOQ	–
	5 × LOQ	–	+	–	3 × LOQ	+++
Bentonite	1 × LOQ	–	–	–	1 × LOQ	+
	5 × LOQ	++	+	+	3 × LOQ	++

this kit because the purified ricin preparation does not contain ATP.

3.3. Impact of powders on kit performance

A sample of each powder was assayed in each kit. The BioCheck® Kit produced positive results for all of the powders containing a proteinaceous component (Table 3). Specifically, the powders that produced positive results using this kit were yeast, DiPel®, dry milk, NIST dust, flour and coffee creamer. The pH of all samples was close to neutral. Using the Prime Alert™ kit, positive results were obtained for powders containing DNA, yeast and DiPel® (Table 3). As expected, Profile®-1 produced positive results for powders containing organisms that contain ATP, specifically, yeast and DiPel® (Table 3). However, two other powders also yielded positive results, flour and talcum powder. Bentonite repeatedly clogged the Filtravette™ filter and results were not obtained for these samples.

3.4. Effect of powders on the agent LODs

The effect of the powders on the agent LODs determined previously was assessed. The agents and powders were mixed and tested with each kit. The BioCheck® Kit detected the presence of proteinaceous powders, therefore, addition of agents did not affect the already positive reading from the kit (Table 4). Several of the non-proteinaceous powders also decreased the ability of the kit to detect agent. However, upon adding five times the amount of agent normally detected by the kit in addition to the powder, a positive result was then restored. This trend was not evident in the case of all powders. This positive result was not found for two of the non-proteinaceous powders. All of the samples had a pH close to neutral.

Powders containing DNA, yeast and DiPel®, presented positive results using the Prime Alert™ kit with addition of agents not altering the already positive outcome (Table 5). Agents were detected in the presence of most powders; only a few inhibited the ability of the kit to detect agent. Addition of increased amounts of agent resulted in eliminating the inhibition.

Using the Profile®-1 test kit, yeast/agent mixtures produced strong positive measurements (Table 6). The powders tested alone that showed positive results using this kit were DiPel®, talcum powder, and flour, while addition of agent did not change the result. For the *B. anthracis* 4× and *B. anthracis* 2×, all powder/agent mixtures showed positive results. For *B. anthracis* 2×, one powder prevented a positive result in the presence of agent. Addition of increased amounts of agent restored the positive result. For the *Y. pestis* /powder mixtures, all of the powder/agent mixtures were positive except

Table 5
Prime Alert™ agent and powder panel testing.

Powder	Agent concentration	Ba 4× wash		Agent concentration	Ba 2× wash		Yp A1122	
		Average reading	Test interpretation		Average reading	Test interpretation	Average reading	Test interpretation
Yeast	1 × LOQ	4141	++	1 × LOQ	3752	++	5930	+++
	1.5 × LOQ	N/A	N/A	5 × LOQ	6384	+++	Over	+++
DiPel®	1 × LOQ	7633	+++	1 × LOQ	9786	+++	8251	+++
	1.5 × LOQ	N/A	N/A	5 × LOQ	Over	+++	Over	+++
Dry milk	1 × LOQ	2278	++	1 × LOQ	2959	++	5406	+++
	1.5 × LOQ	N/A	N/A	5 × LOQ	6438	+++	Over	+++
Powder A	1 × LOQ	1879	+	1 × LOQ	2624	++	4910	++
	1.5 × LOQ	N/A	N/A	5 × LOQ	3653	++	Over	+++
Flour	1 × LOQ	3439	++	1 × LOQ	2725	++	5596	+++
	1.5 × LOQ	N/A	N/A	5 × LOQ	5889	+++	Over	+++
Coffee creamer	1 × LOQ	1975	+	1 × LOQ	2902	++	5138	+++
	1.5 × LOQ	N/A	N/A	5 × LOQ	5449	+++	Over	+++
Salt	1 × LOQ	2389	++	1 × LOQ	2380	++	4854	++
	1.5 × LOQ	N/A	N/A	5 × LOQ	5439	+++	Over	+++
Powdered sugar	1 × LOQ	2672	++	1 × LOQ	2417	++	5206	+++
	1.5 × LOQ	N/A	N/A	5 × LOQ	5538	+++	Over	+++
Talcum powder	1 × LOQ	2485	++	1 × LOQ	2149	++	3847	++
	1.5 × LOQ	N/A	N/A	5 × LOQ	4837	++	Over	+++
Chalk dust	1 × LOQ	2033	++	1 × LOQ	2227	++	3788	++
	1.5 × LOQ	N/A	N/A	5 × LOQ	5137	+++	Over	+++
Foot powder	1 × LOQ	2161	++	1 × LOQ	2411	++	4860	++
	1.5 × LOQ	N/A	N/A	5 × LOQ	4526	++	Over	+++
Powder B	1 × LOQ	1828	+	1 × LOQ	2104	++	5155	+++
	1.5 × LOQ	2110	++	5 × LOQ	5409	+++	Over	+++
Kaolin	1 × LOQ	1359	–	1 × LOQ	1732	+	2941	++
	1.5 × LOQ	1740	+	5 × LOQ	2997	++	Over	+++
Spackling powder	1 × LOQ	1318	–	1 × LOQ	1216	–	2992	++
	1.5 × LOQ	1600	+	5 × LOQ	4378	++	Over	+++
Powder C	1 × LOQ	2204	++	1 × LOQ	2199	+	4015	++
	1.5 × LOQ	N/A	N/A	5 × LOQ	4626	++	Over	+++
Bentonite	1 × LOQ	793	–	1 × LOQ	648	–	535	–
	1.5 × LOQ	1537	+	5 × LOQ	1601	+	2042	+

Table 6
Profile®-1 agent and powder panel testing.

Powder	Agent concentration	Ba 4× wash	Ba 2× wash	Yp A1122
		Test interpretation	Test interpretation	Test interpretation
Yeast	1 × LOQ	+++	+++	+++
	5 × LOQ	+++	+++	+++
DiPel®	1 × LOQ	+++	+++	+++
	5 × LOQ	+++	+++	+++
Dry milk	1 × LOQ	++	++	+
	5 × LOQ	+++	+++	++
Powder A	1 × LOQ	+	+	+
	5 × LOQ	+	++	+
Flour	1 × LOQ	+	+	+
	5 × LOQ	++	+	+
Coffee creamer	1 × LOQ	++	++	+
	5 × LOQ	++	+++	+
Salt	1 × LOQ	++	++	++
	5 × LOQ	+++	+++	++
Powdered sugar	1 × LOQ	++	++	–
	5 × LOQ	+++	+++	+
Talcum powder	1 × LOQ	++	++	++
	5 × LOQ	+++	+++	++
Chalk dust	1 × LOQ	++	++	+
	5 × LOQ	+++	++	++
Foot powder	1 × LOQ	++	++	+
	5 × LOQ	+++	+++	++
Powder B	1 × LOQ	+++	++	+
	5 × LOQ	++	+++	++
Kaolin	1 × LOQ	++	++	+
	5 × LOQ	+++	++	++
Spackling powder	1 × LOQ	++	+	+
	5 × LOQ	+++	++	++
Powder C	1 × LOQ	+	–	–
	5 × LOQ	++	+	–
Bentonite	1 × LOQ	Clogged filter	Clogged filter	Clogged filter
	5 × LOQ			

for one powder, where adding more agent reestablished the positive result.

4. Conclusions

First responders are faced with handling potentially harmful, unknown suspicious powder samples on a regular basis. The responder community requires a rapid mechanism to affordably test these samples for biological hazards and obtain reliable results. Because a void exists for validating suspicious powder screening tools, this study was performed to verify the claims of three generic suspicious powder screening tools, BioCheck® Powder Screening Test Kit (20/20 GeneSystems, Inc., Rockville, MD), the Profile®-1 System (New Horizons Diagnostics, Columbia, MD), and the Prime Alert™ kit (GenPrime, Spokane, WA). These technologies were evaluated for their effectiveness to accurately differentiate between a hoax powder and a true biological agent.

The BioCheck® Kit detected *B. anthracis* spores 4× at a concentration of 1×10^8 CFU, *B. anthracis* 2× at 1×10^7 CFU, *Y. pestis* at 1×10^7 CFU and ricin at an amount of 100 μg (Table 2). A decrease in LOD was evident from the *B. anthracis* 4× to the *B. anthracis* 2× in the LOD testing. This decrease in LOD is not unexpected and most likely results from the presence of proteinaceous cellular debris in the preparation only washed 2× confirmed microscopically. Several substances from the powder panel produced positive results for the BioCheck® Kit including dry milk, NIST dust, flour, and coffee creamer and yeast and DiPel®, both of biological origin. Most of the non-proteinaceous powders minimally interfered or did not interfere at all with the detection of the agents (Table 4). Agent amounts at 1 × LOD, in some cases, were not detected, however, addition of 5 × LOD, restored the positive result. A previous assessment on commercially available urine test strips [2] found that one powder gave false positives for all agents tested however in this study that same powder only interfered with the detection of ricin and *Y. pestis*, but not *B. anthracis*. Another powder affected the ability of the BioCheck® Kit to detect *B. anthracis* 4× and *Y. pestis*. Several overall observations were made for the BioCheck® Kit. The LODs detected were consistent with the manufacturer's claims. The kit was easy to use with clear instructions and did not require additional instrumentation. Five minutes required to perform the assay is considered short for field detection kits and all types of biological agents used in this assessment were detectable. Because this kit relies on generic detection of proteins it will register positive results in the presence of any protein based powders whether these are infectious biological agents or simply bakers yeast. Each test costs $26.20 and does not require any additional equipment to run the tests. In summary the kit was user friendly and accurate in its determination of biological agents.

The Prime Alert™ kit detected *B. anthracis* spores 4× at 2×10^{10} CFU, *B. anthracis* 2× at 1×10^9 CFU and *Y. pestis* at 1×10^8 CFU (Table 2). Ricin toxin was not detected by the nucleic acid component of this kit but the manufacturer has toxin spe-

cific hand-held immunoassays that accompany the kits which are intended to serve that purpose. Those immunoassays were not tested as part of this assessment but the stated detection limit is well below the 100 μg used in these tests. *B. anthracis* 4× had a higher LOD then the 2× sample probably due to the absence of DNA cellular debris with which it could react. The ability of the Prime Alert™ kit to detect lower quantities of *Y. pestis* is most likely due to the ability of the dye to penetrate the membrane of the *Y. pestis* vegetative cells in comparison to the tough spore coat of the *B. anthracis* preparation. Only yeast and DiPel® produced positive results when tested alone with the Prime Alert™ kit but since both powders contain DNA and are biologically active they should not be considered false positives (Table 3). No other hoax powders from the panel presented a false positive result. When mixing the agents and powders together to show powder effects on agent detection, the powder/agent mixtures containing yeast and DiPel® produced positive results due to the presence of the DNA containing powders (Table 5). The presence of most of the remaining powders did not affect the ability of the kit to detect the agent. Three powders did demonstrate a masking effect on the ability of the kits to detect agent. In all cases if the proportion of bio-threat agent was increased relative to hoax powder the kits were able to overcome the masking effect and accurately detect the presence of a biological agent. Overall, the Prime Alert™ kit was easy to use and the total assay time required was less than five minutes. The kit requires the purchase of a hand-held fluorometer in addition to toxin specific immunoassays for ricin and botulinum toxin. Very few powder interferences were observed with the kit. In this study the Prime Alert™ kit was not as sensitive as manufacturer specifications. The LOD determined in this study, $\sim 10^{10}$ CFU, was higher than the manufacturer's claims by two orders of magnitude, 10^8 CFU. The difference in the stated LOD could be due to variations in the spore preparation methods of those organisms. Despite the sensitivity issue the kit was effective in the determination of whether a sample was a biological powder.

The Profile®-1 system detected *B. anthracis* spores 4× and 2× at 1×10^4 CFU and *Y. pestis* at 1×10^6 CFU (Table 2). As expected, this kit did not detect ricin because purified ricin does not contain ATP. The number of washes did not change the LOD determined for the spore preparations. Several powders, in the absence of agent, yielded positive results including yeast (actual RLU), DiPel®, flour, and talcum powder (Table 3). Because both yeast and DiPel® produce ATP and are biologically active, they should not be considered false positives. However, flour and talcum powder do not produce ATP and were categorized as false positives. All *B. anthracis* agent/powder mixtures exhibited positive results, indicating that the powders did not affect the ability of the kit to see biological material. The addition of one powder to the *B. anthracis* 2× wash preparation resulted in a negative result that turned positive in the presence of increased amounts of agent. After the testing of *Y. pestis* had been completed, the manufacturer suggested that the samples should have been incubated for a longer period of time to allow for adequate production of ATP, which would result in a decreased LOD. The protocol used in this study was initially agreed upon by both the evaluators as well as all kit manufacturers for the sole purpose of targeting as many types of agents as possible. Regardless of the elevated LOD for this kit using *Y. pestis*, the kit still produce positive results for the agent in the absence of incubation, clearly indicating that the kit can detect the ATP associated with the bacteria. Almost all of the agent/powder mixtures produced positive results, showing that only one powder could mask the addition of agent. In our hands the Profile®-1 kit was considered easy to use and able to produce low LODs. This kit is capable of detecting any agent that produces ATP, i.e., metabolically active cells. An ancillary portable luminometer must be purchased separately from the individual tests. A few powder interferences were found for this kit that resulted in false positives. An incubation period of 15 min was required for germinating spores and driving them to an ATP-producing state. In addition, some organisms may be metabolically inert and require further incubation to force them to produce ATP. This incubation step may prove to be somewhat undesirable in the field. Although the kit performed according to the manufacturer's claims it would be beneficial if the kit had a single protocol for all suspicious powders and that the methods were clearly articulated for the user community. Additional immunoassay strips are used to detect toxins such as botulinum or ricin.

This study shows that there are several viable detection kits available to first responders that are effective in determining whether a suspicious powder contains biological agents. While there have been several discussions concerning sensitivity of the various kits, it is important to note that if a biological threat agent is present at levels below the LOD of these kits, it will not be discernible by the naked eye and therefore these kits would likely not be employed. Thus, the most important criterion that should be considered is the kit's ability to differentiate non-biological powders from biological powders and rule out many of the powders that cause concern.

Acknowledgements

The authors wish to acknowledge the contribution of Mary Wade for technical consultations, and Todd Sickler, Jerry Pfarr, and Andrew Beck for technical assistance. We would also like to acknowledge the Department of Homeland Security for providing the funding to complete this study. The Department of Homeland Security sponsored the production of this material under an Interagency Agreement with the National Institute of Standards and Technology.

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