Screening Tools for Suspicious Powders: DO THEY WORK?

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EIGHT YEARS after letters laced with *Bacillus anthracis* (anthrax) spores killed seven people across the nation, first responders continue to handle thousands of suspicious powder incidents each year. These incidents disrupt the community, raise anxiety within the population, and cost the taxpayers thousands of dollars.

What today's first responders desperately need is a rapid and reliable method to rule out hoax-powder incidents in order to ease safety concerns and quickly restore commerce. Toward this end, a panel of experts proposed an economical five-step pre-screening kit to rule out suspected biological threats in a powdered form that could be put together with commercially available components and would cost less than two dollars per test. The proposed method relied on measuring specific properties of a suspicious powder—including apparent particle size, solubility in water, acidity, and protein content—to determine whether that powder had the potential to be of biological origin.

Unfortunately, when the method was tested in our labs, we found that although the five-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.

Recently, several commercial systems have been introduced that seek to fill the gap by providing cost-effective biological screening tools that can nonspecifically determine the presence of a biological material in a suspicious powder. The labs at the Department of Defense's Edgewood Chemical Biological Center in Aberdeen Proving Ground, Maryland 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.

BioCheck Powder Screening Test Kit

This system is from 20/20 GeneSystems of Rockville, Maryland. It is a swab-based test kit that utilizes protein detection and pH testing technology for screening powders.

Prime Alert System

This system is from GenPrime of Spokane, Washington. It employs a DNA-based fluorescent-detection technology to determine the presence of bacteria or viruses. 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.

Profile-1 System

This system is from New Horizons Diagnostics of Columbia, Maryland. The system utilizes technology that detects adenosine triphosphate (ATP), a component produced by all living cells. A luciferin-luciferase (LL) reaction occurs in the presence of ATP that is measured by a microluminometer. 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.

Methodology of the Study

The bacteria used in this study—specifically *Bacillus anthracis* and *Yersinia pestis*—were produced using protocols specific for the organism.

Two preparations of *B. anthracis* were produced, each washed a different number of times resulting in varying degrees of residual cellular debris. The first preparation was produced using a plate-harvesting method. It was washed four times during the final steps of the protocol, yielding *B. anthracis* △Sterne 4X. The second preparation was produced using a fermentor with the spores being washed only twice, producing *B. anthracis* △Sterne 2X. The preparation that was washed twice had traces of cellular debris as evidenced under microscopic visualization, while the preparation washed four times showed no traces of the above debris. The *Yersinia pestis* preparation was produced in Brain Heart Infusion Broth and washed two times. Ricin toxin (RCA_{60}) was purchased from the Vector Laboratories.

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, Arm and Hammer baking soda, Domino's powdered sugar, Fleischman's brewers yeast, drywall dust, Morton's iodized salt, chalk dust, Gold Bond medicated foot powder, Ajax cleaning powder, Kaolin, Bentonite, and NIST dust.

BioCheck Kit Test Methods

A known quantity of *B. anthracis*, *Y. pestis*, ricin, powder (10 mg), or a combination of agent and powder was added to the protein-detection tube. The swab containing the detection reagents was added to the protein detection tube and allowed to incubate for five minutes at 77°F (25°C). The same quantity was added to the pH tube. The swab containing the detection reagents was added to the pH tube and allowed to incubate for five min at 77°F (25°C). The color change for both the protein and pH tubes was recorded.

Prime Alert Kit Test Method

Eight drops of Cell Prep Solution was dispensed into the supplied glass vial

The tables referred to in this article can be found in the online version at ***www.EvidenceMagazine.com***

followed by the addition of the Reaction Solution to the glass vial. A known quantity of *B. anthracis*, *Y. pestis*, ricin, powder (10 mg), or a combination of agent and powder was 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 test was performed according to manufacturer's instructions.

Profile-1 System Test Methods

This system integrates an incubation step to allow for the conversion of spores into ATP-producing cells. Therefore, the actual value in relative light units (RLU) obtained by subtracting the unincubated sample from the incubated sample reflects the amount of ATP produced from the agent present, eliminating any background effects. Known quantities of *B. anthracis*, *Y. pestis*, ricin, powder, or a combination of agent and powder were processed using the Filtravette system, bacterial releasing agents, and an extensive wash process. ATP captured on the Filtravette is then measured using the manufacturer-supplied luminometer. 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).

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.

Results

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) 15 powders from the DoD suspicious-powders panel
3) 15 powders from the DoD suspicious-powders panel spiked with one of the pure agents listed 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

Table 1. General Kit Overview

Kit	Manufacturer	Cost per Test	Total Time	Other Equipment Required at Additional Cost	Technology Target
BioCheck	20/20 GeneSystems	$22.80	5 minutes	No	Protein
Prime Alert	GenPrime	$150.00	5 minutes	Yes	DNA
Profile-1	New Horizon Diagnostics	$4.00	20 minutes	Yes	ATP

BioCheck Results

The BioCheck kit detected *B. anthracis* spores 4X at a concentration of 1X108 CFU, *B. anthracis* 2X at 1X107 CFU, *Y. pestis* at 1X107 CFU, and ricin at an amount of 100µg. Cleanliness of a sample affects the ability of the system to detect the agent, because the presence of cellular debris results in a decrease in LOD as seen with the *B. anthracis* preparations.

All of the samples had a pH close to neutral, indicated by the color produced in the pH tube. Several powders from the powder panel, in the absence of agent, produced positive results for the BioCheck kit, including dry milk, NIST dust, flour, and coffee creamer and yeast and DiPel, both of biological origin. All powder samples rendered pH results close to neutral, indicating that pH does not play a strong role in the determination of the biological agent presence.

Most of the non-proteinaceous powders minimally interfered or did not interfere at all with the detection of the agents *(see Table 2 in the online version of this article)*. Agent amounts at 1XLOD, in some cases, were not detected, however, addition of 5XLOD, restored the positive result. This was seen mostly with the chalky powders, where the opaqueness of the powders made it difficult to properly make a positive color determination.

A previous assessment on commercially available urine test strips found that one powder gave false positives for all agents tested. In this study, however, 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* 4X 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. The 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 baker's yeast. The pH assay does not add significant information to the assessment process because all samples—agents and powders—yielded neutral pH values. In addition, when some opaque powders were mixed with biological pathogens, they masked the presence of an agent when the test was conducted at the limit of detection—although a positive result was restored when 5XLOD of agent was added. 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.

Prime Alert Results

The Prime Alert kit detected *B. anthracis* spores 4X at 2X1010 CFU, *B. anthracis* 2X at 1X109 CFU and *Y. pestis* at 1X108 CFU. Ricin toxin was not detected by the nucleic-acid component of this kit, but the manufacturer has toxin-specific handheld immunoassays that accompany the kit that 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 100ug used in these tests.

Cleanliness of a sample affects the ability of this system to detect the agent, in that the presence of cellular debris results in a decrease in LOD as seen with the *B. anthracis* preparations. 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 powders 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. 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 *(see Table 3 in the online version of this article)*. 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 biothreat agent relative to hoax powder was increased, the kits were able to overcome the masking effect and accurately detect the presence of a biological agent. In using the Prime Alert kit, it was found that the system was minimally affected when tested with extremely dense, opaque, and non-soluble powders.

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, ~1010 CFU, was higher than the manufacturer's claims by two orders of magnitude, 108 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.

Profile-1 Results

The Profile-1 system detected *B. anthracis* spores 4X and 2X at 1X104 CFU and *Y. pestis* at 1X106 CFU. As expected, ricin was not detected by this kit because purified ricin does not contain ATP.

Cleanliness of the preparations did not affect the LOD.

Several powders, in the absence of agent, yielded positive results, including yeast (actual RLU), DiPel, flour, and talcum powder. 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 detect biological material *(see Table 4 in the online version of this article)*. The addition of one powder to the *B. anthracis* 2X 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. (Note: 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 produced 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 capable of producing 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 minutes 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.

Conclusions

First responders continue to be forced to deal 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 to 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. 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.

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