SUSPICIOUS WHITE POWDER

Center for Domestic Preparedness develops inexpensive field test for ruling out Anthrax spores.

The Department of Justice, Office of Justice Programs, Center for Domestic Preparedness, Anniston, Ala., (transferred to the Department of Homeland Security March 1, 2003) hosted a working group in late December 2002 to attempt to develop a science- and consensus-based protocol to support emergency response personnel at events involving suspect "anthrax" letters or packages. The Working Group consisted of experienced members from the science community and senior practitioners from the fire service, law enforcement, emergency medical, hazardous materials and responder-education fields (see sidebar). Other scientists, who did not attend the work group, participated in a survey evaluating predictive reliability and value of a series of field and laboratory assays.

With the smoke still rising following the terrorist attacks of September 11, America was faced with another very real threat to its security—the causative agent of anthrax, *Bacillus anthracis*, delivered through the mail. Since 1997, there have been thousands of threat letters containing hoax materials purported to be *Bacillus anthracis* spores, and in fewer cases Ebola, ricin and botulinum toxins. Initially, local responders handled the letters; later the FBI and military laboratories became involved. Eventually, the Laboratory Response Network (Centers for Disease Control and Prevention and American Public Health Laboratories) was developed to deal with suspect bioterrorist samples using standardized procedures.

Until September, 2001, when the letters arrived at the news agencies and Senate offices, none had contained actual *Bacillus anthracis*. The anthrax letters, although relatively few, touched off an even greater number of hoaxes and suspicious packages—called in by citizens concerned for their health and safety—throughout the United States that lasted for months and continue, at a lower rate, even today. Emergency service dispatchers and 9-1-1 operators throughout the country received innumerable frantic calls from citizens who reported the presence of what they were sure was the next anthrax release. Fortunately for our citizens, emergency service providers from jurisdictions large and small responded to these calls. These responders—police officers, firefighters, HAZMAT and emergency medical personnel—arrived at the locations of the suspicious packages and letters, and based on their local protocols or experience, did what they could to successfully resolve the incident. Analytical laboratories processing suspect samples were overwhelmed.

This unprecedented number of responses revealed significant weaknesses in our national preparedness at the local level. Weaknesses that, had more of these letters contained anthrax, could have resulted in more U.S. citizens being exposed to spores and potentially contracting anthrax. In some cases, individuals were unnecessarily quarantined or treated with antimicrobials based on false positive results from unvalidated technologies. Furthermore, the lack of efficient, standardized response procedures and an inability to easily triage the samples increased response costs. Following are some of the more obvious weaknesses and problems exposed by the plethora of hoaxes and suspicious packages reported during the period.

- Standardized emergency protocols for response to potential biological incidents were lacking at the onset of the incidents.
- The large number of responses exceeded the capabilities of even the largest of U.S. jurisdictions.
- Federal policies placing all suspected WMD incidents under the purview of the FBI resulted in delayed on-scene processing and, at times, little support or guid-

Working Group Participants

The Working Group that developed the Anthrax triage recommendations includes:

- Todd Brethauer: WinTec SETA support to Technical Support Working Group
- John Ezzell: U.S. Army Medical Research Institute of Infectious Diseases
- David Franz: Southern Research Institute/University of Alabama at Birmingham
- Michael Hagen: Los Angeles Police Department
- Gary Holt: EAI Corp.
- Robert Hoehl: U.S. Department of Homeland Security
- Larry Kerr: Homeland Security Council
- Brett Lea: Boca Raton (Fla.) Fire Rescue
- Bill Mills: EAI Corp.
- Robert Reid: EAI Corp.
- Michael Sellitto: Washington D.C. Fire & EMS
- Rick Schlegel: EAI Corp.
- Cliff Smith: Sheriff's Dept. Montgomery County, Tenn.

ance for the local response community.

- Mid-sized and small communities lacked even minimal means of analysis to rapidly screen materials in the field to quickly differentiate between a material that was potentially the real thing and other benign white powders used in hoaxes or found by a concerned citizen. Thus, communities had massive resources occupied at incident sites for extended periods, resulting in unwarranted costs in personnel and materials, and unnecessary anxiety for affected citizens.
- Lacking definitive guidance for safely responding to such incidents, some community emergency responders expended unnecessary resources treating each incident as a response to an "unknown, immediately dangerous to life and health," and worked at Level A. [1]
- Communities lacking HAZMAT resources often went to the other extreme, sending emergency responders to the site of the suspicious package with minimal respiratory or percutaneous protection, working at Level D. [1]
- Because of the widespread confusion and conflicting public statements, the public was skeptical of statements made by the emergency response community regarding the personal risk and actions to be taken following potential exposure.
- A gap was exposed in the regulatory framework of the U.S. government that allows certain classes of detection technologies to be sold on the market in an unregulated, unvalidated manner.

The Working Group's objectives were twofold:

1) Determine how a "typical" American community's responders might quickly distinguish a hoax biological event from the real thing, given their current resources; and
2) Develop a consensus field analysis protocol for responding to a potential anthrax letter.

The first objective was selected for a most compelling reason. Local communities—even affluent ones—cannot afford to waste personnel and equipment resources to respond to powdered sugar, especially when there are multiple, concurrent events of this type. Even when they do respond, they simply cannot afford to keep the necessary human and equipment resources occupied for extended periods, awaiting experts and sophisticated identification systems that can determine whether the threat is real or not.

These inexpensive supplies can help first responders rule out the presence of Anthrax quickly and efficiently on scene.

A very practical initial concern for responding agencies is determining expeditiously when an event is not a potential biological event. Doing so would allow agencies to rapidly downsize the response, return to pre-incident levels of manning and operations and ease citizens' minds. Conversely, identifying an unknown substance as biological in origin should elicit an increased awareness that a credible, dangerous threat might be present.

The workshop was based on currently available resources in the typical community; no attempt was made to access or validate commercially available field detection technologies that might be procured and used to support overall detection of suspect agents.

ANTHRAX CHARACTERISTICS

Bacterial, viral and toxin threat agents, such as *Bacillus anthracis* spores, have common characteristics:

- They are all biological materials;
- They typically survive and multiply within a physiological pH range (ca. pH 6.5-8.0); and
- They contain protein and potentially other biological components such as carbohydrates, fats and nucleic acids (DNA and RNA).

A powder such as cleansing agent may have a high pH, greater than pH 9; it also contains no protein. Talcum powder does not contain protein, but its pH may be near neutral. However, other hoax materials might have a physiological pH range and contain protein. Here, particle size or water solubility may differ from that of a threat preparation of *Bacillus anthracis* spores.

Most biological threat agents create a turbid suspension when placed in water; this is true of *Bacillus anthracis* spores. If the material being tested dissolves in water and becomes clear or translucent with no turbidity, it is likely not a spore preparation.

Spore powders are often tan or off-white in suspension and become more cream colored depending on the purity of the preparation. Usually the more pure an agent formulation, the closer to white it becomes. Furthermore, as a general rule, toxin, virus and bacterial preparations become less stable as their purity increases. Therefore, a pure white crystalline material that forms a solution in water is more likely a salt or carbohydrate than a spore formulation.

Selection of the identification methods proposed here was based on these and other fundamental characteristics of *Bacillus anthracis* spore preparations and potential hoax materials. We have selected particle size, water solubility, pH and protein content, in combination, as a potential set of indicators that might help emergency responders evaluate unknown suspect powders.

THE TEST KIT

The Working Group concluded that the following list of supplies is necessary to evaluate—in the field—particle size, solubility, pH and protein content, all variables which might be used to help understand the nature of a dry material found in a suspect letter or parcel.

1) A small clean vial with leak-proof cap. (An approximately 3ml, glass or borosilicate vial can be purchased at medical or laboratory supply stores for approximately $1.65 each.)
2) pH paper test strips. (A pack of 100 with test strip color indicator ("intermediate" range or high-quality "universal" range) can be purchased for approximately $5.)
3) Protein test strips. (A pack of 100 with

TABLE 1: Proposed Biological Field Test System

Test To Be Conducted	Possibly, a Biological (or of respirable size)*	Not Likely to be Biological (or not of respirable size)*
Step 1) Collect two samples: Sample 1 for reference laboratory analysis** ; a volume equivalent to one restaurant sugar packet (ca. 1 gm) or more, if available. Sample 2 for field analysis; a volume similar to the size of a small pea or kernel of corn.		
***Step 2)** Place material to be analyzed in a dry ca. 3ml clean glass vial and secure lid. Shake vigorously for a few seconds and observe.	Fine cloud or haze hangs above sample for several seconds after shaking is stopped	All material falls to bottom of vial, like salt in a salt shaker, after shaking; air above material is clear
Step 3) Remove lid, fill vial ca. two-thirds (ca. 2ml) with distilled water and resecure lid. Shake vigorously for 15 seconds and observe.	Sample appears to mix with water, but does not dissolve. Liquid contents remain turbid or cloudy.	Sample dissolves in water and becomes clear with or without larger particles settling to the bottom.
Step 4) Remove lid of vial and dip pH test strip into water. Remove strip, wait 30 seconds and read result on pH strip container.	pH between 5 and 9	pH less than 5 or greater than 9
Step 5) Remove lid of vial and dip one protein test strip. Remove strip, wait 30 seconds and read result on protein strip container.	Protein is present.	Protein is not present.

NOTE: **The shaking of dry powder in vial as described in step 2 provides only an indication of particle size. This part of the test protocol does not provide an indication regarding the potential for the material being of biological origin.*

*** Standing procedures that address packaging, chain of custody and decontamination for suspect samples should be established with the local FBI in advance of an event.*

TABLE 2: Simple Biological Field Test System Confidence Factors

	VERY HIGH THREAT- Credible threat, same as other confirmed attacks	HIGH THREAT- Credible threat, but no similarities to other confirmed attacks	MODERATE THREAT- Threatening, but assessed as possible copy cat	LOW THREAT- Same as other confirmed suspicious materials	VERY LOW THREAT- Other indications that there is no threat
HIGH RISK LOCATION (Federal or State government offices and courts, major businesses, location similar to other recent confirmed attacks, other hot spots such as transport hubs, women's health clinics or genetically modified organism research centers)	+ Turbidity + Protein + PH	+ Turbidity + Protein + PH	+ Turbidity - Protein + PH Or - Turbidity + Protein + PH	+ Turbidity - Protein + PH Or - Turbidity + Protein + PH	- Turbidity - Protein - PH Or + Turbidity - Protein - PH Or - Turbidity + Protein - PH
MODERATE RISK LOCATION (Local government offices and courts, controversial local businesses or institutions, schools)	+ Turbidity + Protein + PH	+ Turbidity + Protein + PH	+ Turbidity - Protein + PH Or - Turbidity + Protein + PH	+ Turbidity - Protein + PH Or - Turbidity + Protein + PH	- Turbidity - Protein - PH Or + Turbidity - Protein - PH Or - Turbidity + Protein - PH
LOW RISK LOCATION (Private homes, small businesses)	+ Turbidity + Protein + PH	+ Turbidity + Protein + PH	+ Turbidity - Protein + PH Or - Turbidity + Protein + PH	+ Turbidity - Protein + PH Or - Turbidity + Protein + PH	- Turbidity - Protein - PH Or + Turbidity - Protein - PH Or - Turbidity + Protein - PH

test strip color indicators—the most sensitive available—can be purchased for approximately $5.)

4) Small disposable spatula or scoop. (A plastic or stainless steel spatula can be purchased for $3 or less.)
5) Distilled water. (Available at grocery stores for less than $1 per gallon.)
6) Magnifying glass, 10 or 20x. (Optional; can be purchased for approximately $5.)

Responder units should also have the necessary understanding, training, equipment and supplies to safely work in a potentially dangerous environment to package suspect samples for shipment to a reference lab in accordance with regulations for shipping etiologic agents and to decontaminate the area, their equipment and personnel.,[2,3,4]

Table 1 outlines a five-step, proposed procedure for simple field analysis of a suspect sample. The five steps listed—Sample, Shake, Water, pH test, Protein test (SSWPP), can provide the typical American community an acceptable rule-out capability at very low cost—$2 to $6 per test.

INTERPRETING ASSAY RESULTS

A small group of experts concur that this simple five-step biological field test system can provide a "rule out" capability with reasonable confidence (ca. 99%). This test protocol does not have a "rule in" capability; it cannot be used to conclusively identify *Bacillus anthracis*, or other biological agents. Anthrax is not the only biological agent able to harm humans, and thus, the proposed five-step field test would alert the user to other potentially harmful biological threat agents (e.g. smallpox) if they were present. A "possibly, a biological" (non-negative) result from Table 1 cannot be considered positive, due to the broad spectrum of materials that might be found in suspicious letters.

Table 2 can assist responders in evaluating unclear results. The shake test is an indicator of particle size. The results of solubility, pH and protein tests—in whatever combination they are observed—can be evaluated by referencing the confidence factors in Table 2 to ascertain the approximate likelihood that the sample does *not* contain *Bacillus anthracis* spores.

Scores in the green zone are considered very unlikely to be a biological threat, and may be treated as such. Scores in the red zone should be considered potentially dan-

TABLE 3: Field Test and Reference Laboratory Assays: Rule-in/Rule-out Potentials

Test	Rule IN Power	Rule OUT Power
In the field:		
1) Visual exam + Dry powder in vial and shake Provides some sense of particle size, thus inhalation threat	20% (0-40)	46% (20-70)
2) Dissolve in water	30% (10-50)	70% (60-80)
Turbidity	Cloudy	Clear
pH	Between 5-9	Below 5; above 9
Protein	Yes	No
Advanced Technologies:		
3) Phase Microscopy Wet mount –spores only; need at least one million spores	52% (30-70)	74% (60-90)
4) Laboratory immunoassays e.g. ECL, ELISA, FA	78% (60-90)	82% (70-100)
5) PCR of sample directly Polymerase Chain Reaction	86% (80-100)	92% (80-100)
6) Culture Plus Phage, DNA extract & PCR and Biochemistry	96% (90-100)	96% (90-100)
7) Total Genetic Sequence	100%	100%

NOTE: *This table of estimated assay power is based on input from five laboratory scientists involved in identification of Bacillus anthracis and other threat pathogens. Data are depicted as "mean % (range)". Definitive identification of biological threat agents, especially for forensic purposes, is a complex and equipment-intensive process. The "In the laboratory" portion of this table is provided to give emergency response professionals a better sense of this complexity and an understanding of the importance of close collaboration with our developing reference laboratory system.*

gerous and treated as such. Scores in the amber and yellow zones should also be considered potentially dangerous, of high and moderate threat, respectively.

The tests are particularly useful when integrated with current intelligence/operational threat condition and knowledge of potential targets in the local community. "Level of threat" at the top of the column captures that intelligence/operational threat information (whether other attacks/confirmed hoaxes have occurred recently). Note that "location of the incident" in the community may be used to further assess the threat; these categories are listed along the left column of the chart.

SUBSEQUENT ANALYSIS

The test and confidence factors described in Tables 1 and 2 are proposed to assist emergency responders in making decisions regarding the relative threat of an unknown powder sample to their citizens. Definitive analysis and actual identification of an unknown can only be done with much more complex instrumentation in a reference laboratory. As part of this initiative, the authors sought the guidance of a number of scientists in an attempt to estimate the "power" of the simple tests described above, and of a more complex set of laboratory tests for ruling in (it *is* anthrax) and ruling out (it *is not* anthrax). The results of a blind survey of experienced laboratory scientists are depicted in Table 3. The power of each assay class was rated from 0-100%, with 0% being of no value in discriminating and 100% being a definitive result.

OUR CONCLUSIONS

The Working Group concluded that it is possible to rule out *Bacillus anthracis* spores in a suspect letter or package, in the field, with relatively simple and inexpensive equipment. The group proposes a simple five-step process involving a few minutes and a few dollars worth of equipment and materials. We believe that the system described here has the potential to provide emergency responders in every community in America the tools to determine, with an acceptable degree of confidence, that an unknown sample does *not* contain primarily *Bacillus anthracis* spores.

As the federal government continues to standardize and broaden its laboratory system to respond to unknown samples and augment the Laboratory Response Network, we may one day have Specimen Triage Units in many cities. Until that time—and even after—we believe the local responder must be given the best and most cost-effective tools we can provide to make a very difficult job somewhat easier. It is our hope that the concepts presented here will do that.

This simple protocol *cannot* determine if an unknown sample *does* contain *Bacillus anthracis* spores. The authors believe that, by increasing their confidence that potential suspicious materials reports are unfounded, responders in communities across America will be better able to make informed decisions regarding the use of resources, thus reducing the human and material costs of protecting our citizens. HFR

For more information, email David Franz at *da.smith@sri.org*.

References

[1] Title 49 Code of Federal Regulations Part 172, Department of Transportation, Hazardous Materials Table, Special Provisions, Hazardous Materials Communications, Emergency Response informationtion, and Training Requirements.

[2] *www.iata.org*

[3] *www.dot.org*