Dr Christina Baxter, of EmergencyResponseTIPS.com, is joined by a special guest to relook at old cases

Lessons worth sharing

This series takes a fresh look at historic events to see how responses would be different today, using what we have learned, and new technologies. Here, we focus on the 2001 attacks in which Bacillus anthracis (Ba) was sent through the post.

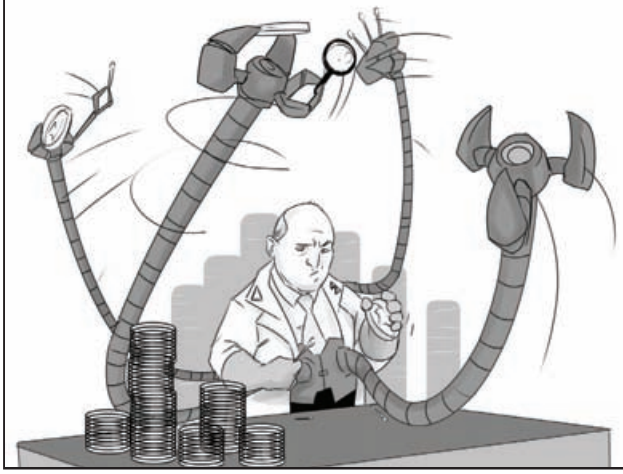
A total of seven letters containing Ba were mailed in two waves, 22 people developed symptoms, 11 had inhalational anthrax and five died. The spores contaminated everything as soon as the letters were posted, and sorting stations, postbags and the offices had to be sampled and decontaminated. The wide variety of locations, and the technology available, made it an extremely challenging case.

Dr Douglas Beecher was among the FBI laboratory staff that had to deal with the incident.

Presidential Decision Directive 39 (PDD-39) formalised the US government's response plan too terrorism including WMD in 1996, and named the FBI as lead federal agency for investigating crimes involving CBRN materials. That year the FBI Laboratory's hazardous materials response unit was created to handle such incidents. I joined the unit in July 2001 as a microbiology subject matter expert (SME) and operational microbiologist. My first year was spent training with the lab and the FBI's 27 hazardous materials response teams, learning about FBI's partnership with the Centers for Disease Control and Prevention and Laboratory Response Network and attending exercises. Mostly, we would arrive at a biological crime scene and try to figure out the cause, usually by sending samples to a lab.

Then, dried Ba spores were mailed, infecting people and killing five. I was deployed to the first crime scene. We already knew the cause, and quickly learned that the point where our exercises always ended was actually the very beginning of what would become one of the FBI's longest investigations. I also learned my most important lesson thanks to an operational breakdown - biological sampling operations work best when the laboratory is fully integrated into the overall system.

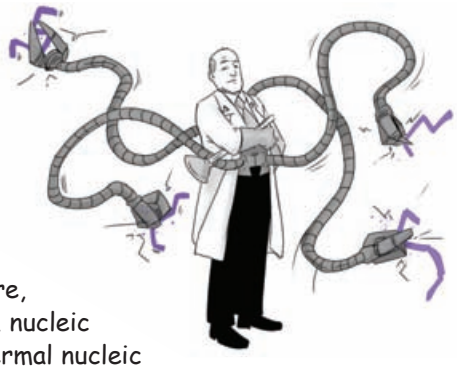
At that first scene we initially knew of one victim and a few positive samples from his workplace. If we were to understand how victims were exposed, we would need to thoroughly sample the 65,000ft2 (6,040m2) building and the results from one day would guide actions on following days. But lab results trickled in so slowly that on-scene operations nearly ground to a halt. We left the site after almost two weeks still not knowing what happened in the building and having to infer that contaminated mail was involved, based on developments elsewhere.



The main problems were that our lab was almost 600 miles away, operating as a separate entity under the standard laboratory business model and viewing us a customer. It operated on its own terms, using standard public health assays for forensic samples and following pre-established reporting policies, all of which were detrimental to our sampling operation. The assays included a three day culture-based method and a rapid PCR method that was not then considered 'gold standard'. Multistep labour intensive setup processes overwhelmed a limited analysis workforce faced with too many samples, so that even the 'rapid' PCR process became bottlenecked. Only confirmed positive results were reported, no negatives, and no preliminary results, all of which were important for us.

Lessons Learned: Amerithrax

I developed an alternative sampling system to overcome gridlock by shifting the burden of assay setup to the on-scene sampling personnel and fully integrating the lab into the operational structure. Sampling personnel inoculated Petri dishes, which were transported to a local laboratory for overnight incubation. Analysis personnel interpreted growth in the morning and reported preliminary results by 9:00am to the on-scene SME. Confirmatory testing on presumptive positives was completed while field operations continued with knowledge of the previous day's results. The system contributed to successful multi-day sampling operations leading to critical evidence and an understanding of what occurred at the crime scene.



The culture assays we used worked because of a favourable combination of factors. Dried Ba pores are highly stable and remain culturable for long periods making their contamination patterns persist throughout the investigation. The surfaces of interest were relatively free of bacteria that would interfere with Ba cultures. And the spores grew readily and quickly on simple to inoculate, commercially available culture media. Most other biological threat agents are less stable and harder to grow on culture, so a detection system that doesn't rely on viability is needed. Fortunately, nucleic acid detection technology has come a long way and some of the new isothermal nucleic acid amplification technology methods may be adaptable for setup in the field and completion in the lab. With proper engineering it may be possible to produce an assay in which the sampler places a swab in one tube containing all necessary reagents that is transferred to a local, mobile, or forward modular lab where the reaction is initiated at its running temperature and the qualitative or quantitative results recorded.

The Amerithrax incident clearly demonstrated that events do not happen in isolation. What started as a white powder letter evolved into multiple crime scenes over the course of the investigation – one of the longest in FBI history - and much became evident early in the investigation.

Training and Exercises

Training and exercises must be multi-agency incorporating complex, yet credible events. The scenarios should be geographically dispersed when dealing with coordinated attacks to ensure that the complexity of command and control is challenged. They must also address tactical, operational and strategic objectives including the collection, decontamination, packaging, and transport of samples, chain of custody paperwork, field clearance and laboratory analysis of samples, decision making, communication, logistics and more.

Unfortunately, many exercises today end at the target identification and initial sample collection phase, and do not follow through for the initial analysis and feedback to field operations to further drive events. This critical gap was very clear during the Amerithrax investigation and in other incidents over the years, due to an operational disconnect between field and laboratory operations.

Sampling

The ability to collect biological powders safely without aerosolisation is problematic when weaponised samples are involved. If there is little concern about sample aerosolisation, standard sampling techniques that transfer samples into a vial for later analysis are acceptable. When dealing with potentially weaponised biological agents, however, the concern for aerosolisation is heightened. In these cases, consider other approaches such as vacuum sampling using the Bulk Particle Collector from Seacoast Science, Inc where the sample is vacuumed directly into the sample vial and is sealed for evidentiary purposes. [See *CBRNe World* October 2020 for further details.]

Field Detection

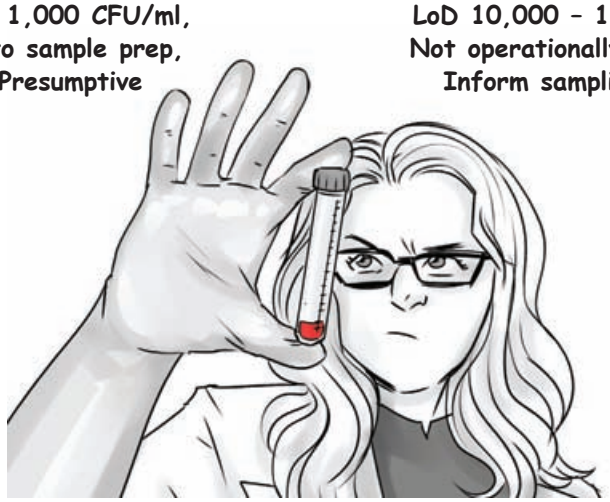
Field detection technologies for biodetection have advanced greatly in the past 20+ years. Where intelligence indicates a material may be a biological threat, however, the priority must be to preserve a sample for laboratory analysis. For powder samples, collect both a solid sample and a wet sample for evidence. A few milliliters of sample are sufficient for laboratory analysis when you consider that there can be upwards of 108 anthrax spores in a ml of solution which represents more than 10,000 lethal doses.

It is important to remember that all field detection technologies are considered presumptive for legal purposes. They should only be used to minimise operational risk, help inform selection of sampling sites, and evaluate the effectiveness of decontamination and cleaning, so a site can be safely reoccupied when awaiting laboratory results. While it might be tempting to use the simplest of biological detection assays, they are not sensitive enough. Remember that ATP tests cannot detect spores unless a spore germination step is added, while protein tests cannot detect less than approximately 10m spores.

Handheld immunoassays (HHAs) range in their ability to detect anthrax from between 10,000 and 1m colony forming units (CFU)/ml. Putting this into perspective, an infectious dose of anthrax via inhalation is around 10,000 spores or less, so while HHAs have a role in field operations, they cannot detect anthrax at operationally relevant concentrations.

PCR (new)
LoD 1,000 CFU/ml,
Auto sample prep,
Presumptive

HHA
LoD 10,000 – 1m CFU/ml,
Not operationally relevant,
Inform sampling sites



Field based PCR assays can detect around 1000 CFU/ml making them more suitable for analysis of threats at operationally relevant levels, however they have limitations and cannot distinguish between viable and non-viable biological threats. New commercial systems, such as the Biomeme Franklin ISP and the BioFire FilmArray, automate much of the sample preparation to minimise cross contamination and errors. While these systems include sample blanks and standards, they are still considered presumptive unless performed in a laboratory. Lab analysis remains the gold standard.

Decontamination

The clean-up following the Amerithrax events cost more than a $1bn. Solutions based on bleach, acidified bleach, dichloroisocyanuric acid and peracetic acid have all demonstrated effectiveness in destroying anthrax spores. Chlorine dioxide was also successful. Be sure to remember that the concentration of spores, spore location, concentration of active decontamination ingredient, environmental conditions (temperature and humidity), presence of organic materials, building material/environmental media and contact time all play a significant role in efficacy.

Most importantly, ensure that your plans are contemporary and include all partner agencies before you need to employ them. Practice tactical, operational, and strategic actions to minimise pitfalls during an actual operation.

Images are courtesy of Phil Buckenham *https://philbuckenhamart.wixsite.com/philbuckenham*