

BIOHAZ: BIOLOGICAL DETECTION FOR EMERGENCY RESPONDERS

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ABSTRACT

There exists an urgent need for emergency responders to rapidly detect the presence of biological materials in a suspect sample. The BioHAZ™ Kit has been recently developed to fill such a need. It is intended to give the emergency responders an integrated capability to collect an environmental sample and to rapidly screen that sample on site for the presence of biological material. This allows the incident commander to be aware of a potential biological hazard and call for the proper response to the situation.

This kit consists of both sampling and detection equipment. It has a variety of sample collection and processing packets to obtain solid, liquid, or air samples. Samples are then screened using multiple, complimentary technologies that are well-proven to determine the presence of biomarkers such as ATP, DNA, and protein within the samples. A simple algorithm allows the operator to determine if the sample may contain bacteria, spores, or protein. Samples can then be further analyzed on site with immunoassay tickets before being sent to a laboratory.

This system provides the emergency responders with a capability that they do not have. The adoption of this kit could result in savings of time and money by debunking biological hoaxes and providing timely warning of the presence of actual biological materials. The BioHAZ™ Kit may also deter the use of biological agents when used as part of a system to mitigate the effects of a biological incident.

INTRODUCTION

As the result of an increasing number of bioterrorism hoaxes and incidents throughout the United States, there is a recognized deficiency among the emergency response community to rapidly detect the presence of biological materials at the site of an incident that is suspected of involving a biological agent. In the late 1990s, the U.S. Army Edgewood Chemical and Biological Center initiated an effort to develop an expedient capability for first responders to sample, detect, and identify the presence of hazardous biological materials in the environment.

The first step in this effort was to identify user requirements and to develop a concept of operations for the use of the kit. Design goals and performance specifications were then established with assistance from the responder community to develop the first prototype, called the Biological Detection Kit (BDK). The performance goals of the BDK were adopted from the first generation Biological Integrated Detection System (BIDS) that is currently fielded by the US Army for biological detection. The requirements of the kit were that it should be able to collect all forms of samples, but work primarily with surface samples to answer the question: “Does that device contain a biological material? If it is bacteria,

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is it viable?" Additional goals of the development effort were that the total cost of the kit should not exceed \$20,000 apiece, that all of the components had to be commercially available, and that the entire kit had to be portable.

DEVELOPMENT OF THE BIOHAZ KIT

MARKET SURVEY AND APPROACH

A market survey to identify technologies that would meet the mission requirements of the BDK was performed and, upon completion, the problem was re-evaluated. It was then decided to change the focus of the effort. The original concept consisted of a kit with sampling devices and a library of immunoassay test tickets for identification purposes. The concept was changed to a more generic approach for a variety of reasons, some of which were:

1. A limited library of test tickets capable of identifying "military threat" biological agents is available. The tests may miss other pathogenic biological materials that could be used by a terrorist but might not otherwise be thought of as a battlefield biological agent.
2. Immunoassay test kits have to be used within concentration and pH guidelines. Tests of samples that are outside of these guidelines may give erroneous results. It would be more useful for field analysis to have the means to determine if the sample was within these guidelines necessary for accurate use of the test kits.
3. Genetic engineering of organisms or other treatments may compromise the ability of antibody-based or nucleic acid probe-based tests to detect and/or identify pathogenic materials.

Based on the market survey, a BIDS-like approach was then adopted in which multiple, complimentary technologies are used as generic tests for biological traits. By comparison, the BIDS uses a particle counter to measure fluxes in the quantity of threat sized particles (1-10 μm) in the environment. Upon alerting to a spike in the particle flux, the BIDS operators collect a sample and analyze the sample for adenosine tri-phosphate (ATP) content and for deoxyribonucleic acid (DNA) content. If the sample possesses certain criteria, it is subjected to immunoassay techniques for further analysis. This scheme is based on the:

1. Particle size and count determination. Most intentionally man-made particles that have viable organisms and/or biological activity are typically above three microns (3 μm) in diameter.
2. ATP determination - detects the presence of living things.
3. DNA test - most all biological materials have DNA. Although purified proteins are assumed to contain no DNA, they are often contaminated with sufficient residue DNA from the protein source to be detectable.
4. Immunoassay test - the antibody-antigen reaction of immunoassay tests give a confirmation of the presence of specific biological agents within the available library of tests.

Tests similar to those are used in the BIDS are used in the BioHAZ kit, but because of cost, size, and weight constraints, the approach on how these tests are used was changed. For example, the BIDS uses a flow cytometer to do DNA detection. This instrument is capable of distinguishing between spore and vegetative cell DNA. The cytometer weighs 300 pounds and has a price tag of \$100,000. The BioHAZ approach will detect DNA in the sample using a hand-held fluorometer, but does not distinguish what type. The BioHAZ luminometer is similar to the one in the BIDS. The BIDS luminometer measures total ATP and cannot differentiate among non-bacterial ATP, vegetative bacterial ATP, and spore ATP. The model used in the BioHAZ Kit has the capability to differentiate among these sources of ATP. Therefore, the BIDS uses the cytometer to differentiate among types of biological material and the BioHAZ Kit does it with the luminometer. Unlike the BIDS, a generic protein detection capability was added to the BioHAZ Kit for detection of proteinaceous toxins, such as Botulinum toxin and Ricin. Also, the design of the BioHAZ Kit eliminated the use of the particle counter used in the BIDS. With the BIDS, this instrument is used as a real-time aerosol sampler to trigger further actions of sample collection and subsequent analysis. The use profile of the BioHAZ Kit presumed that, if a biological attack had

occurred in conjunction with an incident that resulted in an emergency response, any biological materials disseminated as an aerosol would have precipitated onto surfaces from which they can be collected using the surface sampling components of the kit.

Since there are a variety of sampling systems in the commercial marketplace for use in different applications, it was decided to integrate some of these into the kit for specific purposes. Individually sealed, expendable sample collection kits were developed to enable the user to collect environmental samples in various forms. Specific kits are included to collect samples from large surfaces (Swipe-1 Kit), small surfaces (Swipe-2 Kit), liquids (Swipe-3 Kit), and from air using filter systems designed either for air sampling or asbestos monitoring (Swipe-4 Kit). In addition, sample processing kits (SPK) were added to allow the user to refine the collected sample for testing on site.

TECHNICAL CONSIDERATIONS

Hand-held particle counting instruments. Small, hand-held particle sizers, including: the Met-One models 227A (2 channel) and 237A (6 channel) and the Bio-Test AG (4 channel) were obtained and evaluated for use in the BDK. They were tested extensively during several joint field trials held in the U.S. They compared relatively well to some of the more expensive instruments currently available. As the approach for the BioHAZ Kit differed from that of the BIDS and the original BDK development, the use of a particle counter Kit was eliminated from further consideration.

ATP luminescence detection instruments. Three luminometers were evaluated for detection of bioluminescence: the IDEXX Lightning System, the New Horizons Diagnostics Model 4700, as it is used in the BIDS, and the New Horizons Diagnostics Model 3550. Although other systems exist and are mainly used in food safety and sanitation monitoring, these three units offered some distinct advantages. The IDEXX system has all of the reagents packaged in a swab device. The Model 4700 is used in the BIDS and would be representative of some of the other luminometers that read total ATP. The Model 3550 has a separation step that allows the operator to eliminate non-bacterial sources of ATP. This process was further used to develop a test capable of detecting spores. This test could not be easily achieved with the other instruments. The New Horizons Diagnostics Model 3550 was therefore implemented for the following reasons:

1. Tested by the USDA and several other organizations ^{1, 2, 3} under field conditions and found to be the only luminometer that showed good correlation with culture results.
2. Approved by the FDA for detection of bacteria in human urine.
3. Used by several commercial companies to measure bacterial contamination in process control measurements.
4. Shown to be the least prone to interferences.
5. Allows the operator to use a variety of sample volumes for analysis.

DNA detection. For DNA determination, the Hoefer DNA Quantitation Kit from Pharmacia Chemicals, Inc., was initially evaluated. The instrument analyzes in the UV. It was not sufficiently rugged to be of use in the field and the dye was highly prone to interferences from the sample that could not be controlled. The PicoGreen dye kit from Molecular Probes was also evaluated ⁴. This kit is readily available and gives detection limits at the required level. It is routinely used in reference labs to quantify DNA in samples prior to PCR analysis. It does not seem to be prone to matrix interferences and has a related dye that detects RNA (single-stranded nucleic acids). To utilize the PicoGreen dye, the Turner Designs TD-360 fluorometer was initially considered for the BDK. It performed well within the required detection limits. The BioHAZ Kit incorporates the newer, smaller Turner Designs instrument, TD-00, which operates at the same performance specifications as the TD-360.

Protein detection. The Coomassie Blue protein test was initially evaluated, since it was the only protein test that met the time and other mission requirements. Other tests either took one hour or required boiling of the sample. Interference from detergents was found with this test. Field experiences with the test showed that an adoption of a cut-off of 10 µg/ml of protein from surface samples was necessary to minimize false positive test results. Likewise, a value of 2.5 µg/ml protein was chosen as the cut-off

value for air samples. Several colorimeters were evaluated for use, types that are used for water analysis and typically range in price from \$200-600. The ChemMetrics VVR colorimeter was initially accepted for use with the BDK. An improvement with the BioHAZ Kit is the replacement of the Coomassie Blue reagent and the colorimeter with the colorimetric protein test strips commonly used for urinalysis. Although the detection limit is increased to about 50 µg/ml with Bovine Serum albumin, these strips are less prone to interference, far less expensive, and easier to perform than the Coomassie test.

Instrument integration. At the conclusion of the laboratory development phase, the instruments were given to emergency responders for field trials. Based on their experience and advice, it was decided that an integrated instrument housing both the luminometer and the fluorometer functions should be built. The resulting instrument is depicted in Figure 1. Since this dual instrument consists of the Model 3550 luminometer and the TD-00 fluorometer components in a single outer housing, the performance of each instrument is the same as the original components.



Figure 1: BioHAZ instrument.

SUMMARY OF TESTING RESULTS

A variety of tests were performed both in the laboratory and in the field ⁵. The luminometer was evaluated with five different types of bacteria, including gram positive spores, cocci, and bacilli, and gram negative bacilli and cocci. Laboratory tests showed that using the BioHAZ system protocols, the luminometer could routinely detect 10⁵ colony forming units of vegetative bacteria per milliliter of solution (CFU/ml), 10⁶ CFU/ml of spores using the spore test procedure, and could differentiate between spore forming and non-spore forming bacteria. This was demonstrated during a recent joint field trail in the U.S. Interferences from common household, laboratory, and environmental chemicals were also evaluated. The ATP test showed no interference from any of these materials, although timing was crucial as to when the sample was tested relative to when it was collected. The DNA test did exhibit false positive results when tested with phosphates; however, in the scenarios under which the kit would be used, this should not be a problem since the presence of phosphate may be another indicator of biological material. The urinalysis test strips were not subject to interference from these materials.

The results in Table 1 list the performance capabilities of the BioHAZ Kit for each test. Table 2 shows a comparison of the BioHAZ Kit to the BIDS.

TABLE 1: BioHAZ testing performance capabilities.

Original Goal	ATP Test (1)	DNA Test	Protein Test
Bacteria - 5x10 ⁵ CFU/ml	<10 ⁵ CFU/ml (vegetative) ~10 ⁶ CFU/ml (spores)	~10 ⁵ CFU/ml (vegetative) ~10 ⁶ CFU/ml (spores)	N/A
Viruses - 10 ⁶ PFU/ml	N/A	~10 ⁷ PFU/ml	N/A
Toxins - 500 ng/ml	N/A	See note (2)	~50 µg/ml

Notes: CFU: Colony forming units; PFU: plaque forming units.

(1) The ATP test measures only live bacteria. The DNA test can measure the presence of either live or dead bacteria. It was also noted that the signal increases, hence the detection limit decreases, if detergents are used to disrupt the bacteria.

(2) At protein concentrations greater than 10 µg/ml, there is detectable DNA present that is typically 1/1000th of the protein concentration.

TABLE 2: Comparison between BIDS and BioHAZ Kit.

Specification	NDI BIDS	BioHAZ Kit
Measure changes in biomass	Measure total ATP with Model 4700 luminometer	Measure change in DNA concentration with fluorometer
Differentiate vegetative cells and spores	Uses DNA dye with flow cytometer	Measures ATP flux with luminometer after incubation
Bacterial detection limit	5×10^5 CFU/ml	$< 10^5$ CFU/ml
Generic detection	Bacteria, probably virus, no protein	Bacteria, probably virus, protein
Cost	~\$1,300,000	\$20,000
Size and weight	1-1/4 ton HMMWV (~12,500 lbs.) with trailer (generator)	~ 50 lbs., ~5 cu. ft. total

These results indicate that the BioHAZ Kit sufficiently meets the specifications to do what it is designed for, to indicate the presence or absence of biological materials, and it does so for a much lower cost than the BIDS, after which it was designed.

USING THE BIOHAZ KIT

The emergency responders at an incident may be called upon to use the BioHAZ Kit. There are several reasons or scenarios that might prompt an Incident Commander to initiate the use of this kit, but the end result will be to provide additional information about the possibility of biological materials being present at the incident scene. Responders and other operators can use the kit to check specific locations for suspected agents (e.g., the material inside of an anthrax letter or the area surrounding the release of a suspected biological agent), survey areas of interest for hot spots, determine the extent of contamination, measure the relative effectiveness of decontamination operations, monitor water, or for other inspection purposes. If the kit indicates the presence of biological materials, this information can help to:

1. Determine the extent of additional response required. For example, there may be requirements for protection, decontamination, or evacuation of potentially exposed personnel for medical evaluation.
2. Determine the type of biological materials that are present. This information can be passed to supporting medical facilities to help them decide on treatment protocols for the victims and to supporting laboratories to narrow their efforts in identifying the specific biological agent used.
3. Determine the type of immunoassay that can be performed on site. The results of these assays help to identify or eliminate specific biological agents from further consideration.

If the BioHAZ Kit tests indicate that no biological materials are present, the Incident Commander can scale back the response efforts toward termination of the incident. This is the case with the responses to hoaxes, such as the ubiquitous anthrax scare letters.

Using the BioHAZ Kit is a simple process of collecting the samples, processing them for testing, testing the samples for biological materials, and evaluating the results for further action. Sample collection is facilitated by the use of individually sealed collection kits. One example is pictured in Figure 2. Once the sample has been collected, the operator can elect to forward the sample to a federal agency, or to test it on site with the BioHAZ Kit testing components. The kit contains the necessary components to package samples for transfer off site and to process samples for testing on site. A sample to be tested is refined into a clear, aqueous solution suitable for testing by using the SPK. The operator then performs quick tests using the instrument and the test strips. Table 3 indicates the type of



Figure 2: Swipe sampling kit.

results that can be expected from using the test equipment of the BioHAZ Kit. Depending on the results, the operator can perform assays specific to the type of biological materials found in the sample. For example, if toxin were likely to be present, assays for toxins (e.g., Ricin, etc.) would be used in lieu of other available assays. So too, if spore-forming bacteria were found, the appropriate assay (e.g., anthrax) would be warranted.

TABLE 3: Expected results of testing.

Indicates Sample Contains	ATP Test	DNA Test	Protein Test
Bacteria	Positive	Positive	See note (1)
Virus	Negative	Positive	See note (1)
Toxin	Negative	See note (2)	Positive

Notes:

- (1) Possibly present from culture media or lysed cells.
- (2) If protein concentration is above 10 µg/ml, will probably detect contaminating DNA.

The final results of any field testing must be verified by a laboratory. Even if an immunoassay indicated the presence of a specific biological agent, these results must be confirmed for positive identification. If other agents not detected by the available assays were used, the BioHAZ Kit will detect and categorize their presence but not provide any further information. For emergency response purposes, this indication alone is sufficient to determine the next series of response actions.

CONCLUSIONS

The BioHAZ Kit successfully does exactly what it was designed to do. It can provide emergency responders and other users the means to rapidly screen and determine the operationally significant presence or absence of biological materials in suspect samples.

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