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BaDx: Portable Diagnostic Device for Dangerous Pathogen Detection in Low Resource Environments

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Worldwide Prevalence of Anthrax and Risk of Theft

Anthrax poses a significant threat to National Security as demonstrated by the 2001 terrorist attacks targeting the US Postal Service and Hart Building. The causative agent, *Bacillus anthracis (B. anthracis)*, is ubiquitous worldwide. More importantly, it is found in countries harboring terrorists.

Anthrax outbreaks commonly occur in livestock. Consequently, the agent is routinely isolated, propagated, and maintained in laboratories (often with no security) by indigenous populations to diagnose the disease. This practice drastically increases laboratories' repositories of *B. anthracis* and escalates the risk that the agent can be stolen for nefarious purposes. Moreover, it enhances the capabilities of laboratory personnel to produce pure *B. anthracis* isolates.

"Deadly diseases like Ebola, Marburg, and Anthrax are prevalent in Africa. These pathogens can be made into horrible weapons aimed at our troops, our friends and allies, and even the American public. This is a threat we cannot ignore." -*Senator Lugar,* stated during his 2010 trip to Africa

Anthrax Outbreaks in 2011

Between 2005-2012, there were 3057 Anthrax outbreaks reported.

This is likely an underestimation of the incidence and prevalence of the disease.

Collection & Identification of Dangerous Pathogens

Melissa Finley trips to Iraq and Afghanistan
Jason Harper trips to Conakry, Guinea

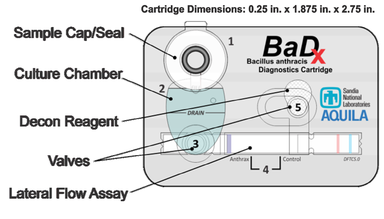
Development of a Sustainable Anthrax Diagnostic Assay

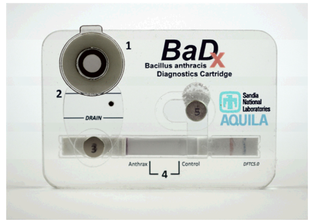
Objective: *Develop a portable diagnostic device for B. anthracis for use in developing countries where biological threat proliferation is a serious concern*

Device Requirements

- Field-deployable
  - *Approximately credit card sized*
  - *Rugged/robust*
  - *Withstand high temperatures*
  - *No cold-chain (refrigeration) requirement*
- Very low cost (< 5 USD)
- Self-sterilizing upon completion of assay
- Detection limit ~100 spores
- No power to operate
- No instrumentation or equipment required to operate/read
- Operable by individuals with little or no technical training
  - *Target: Operation & read-out by individuals with 3rd grade education*

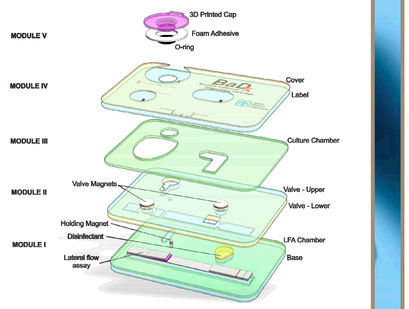
B. anthracis Diagnostic (BaD_X) Cartridge





Microfluidic platform for bacterial detection prepared from laser ablated plastic laminates

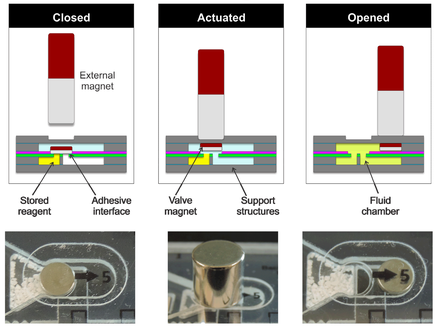
Anthrax Detector Modules and Functional Components



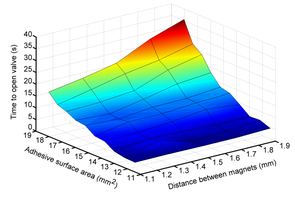
BaD_X Anthrax Detector

- Selective bio amplification (micro-culture)
- One step lateral flow assay for detection
- On-device sterilization (chemical decon)
- External magnet actuated valves
- Materials cost: $12

Novel Magnetic-Adhesive Valves

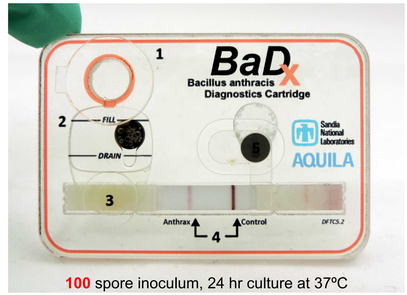


Surface plot of the average time required for magnetic-adhesive valve actuation



- *No power required*
- *No pumps*
- *Allows on-device reagent storage*
- *Simple to operate*
- *Maintains seal*
- *Low cost ($0.40 each)*

< 10 Spores *B. anthracis* (Ames) Detected



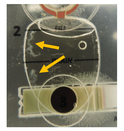
100 spore inoculum, 24 hr culture at 37ºC

BaDx detection with varying *B. anthracis* inoculum

Initial Spore Inoculum	Positive at 15 minutes	Positive at 60 minutes	Positive overnight
1000	5/5	5/5	5/5
100	3/5	4/5	5/5
10	2/5	4/5	5/5
5	0/5	2/5	3/5
3	0/5	1/5	3/5
1	0/5	2/5	3/5

- *This is a 4-5 order of magnitude improvement in detection limit over LFA alone*
- *Brings detection limit within a practical range for real-world samples*

Selective Amplification



Dual-selectivity:
- Selective growth medium (PLET++)
- LFA selectivity (TetraCore RedLine LFA)

Highly reliable biodetection in the field, rivaling the selectivity of laboratory analysis

Specificity of BaDx inoculated with 10^5 cfu of the given bacteria

Result	*Bacillus thuringiensis*	*Bacillus cereus*	*Bacillus subtilis*	*Escherichia coli*	*Francisella tularensis*	*Streptococcus pyogenes*
Samples (n)	11	10	12	10	12	12
Negative	10	10	12	10	12	12
False Positive	1	0	0	0	0	0

Complex sample matrices spiked with 10^4 *B. anthracis* spores in BaDx

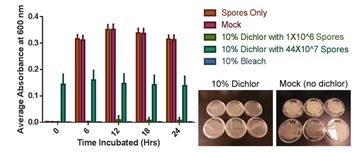
Result	Dirt	Whole Milk	Mouth Swab	Nose Swab	Human Serum	Horse Mouth	Horse Nose	Stable Soil
Samples (n)	5	6	5	5	5	5	5	5
Positive	5	5	5	5	5	5	5	5
False Negative	0	1	0	0	0	0	0	0

Post-Assay Self-Sterilization

Self-decontamination following assay greatly minimizes the potential for malicious use of the bacterial sample. It also reduces the need for experienced technicians to isolate the organism in the laboratory, reducing laboratory stores.

On-device chemical decontamination: sodium dichloro-s-trazinetrione (dichlor)

Most Extreme Condition Tested: *100% Spores [1 x 10^6]*



Publications and Patents

1. Harper, J. C., Andrews, J. M., Ben, C., Hunt, A. C., Murton, J. K., Carson, B. D., Bachand, G. D., Lovchik, J. A., Arndt, W. D., Finley, M. R., Edwards, T. L. "Magnetic-Adhesive Based Valves for Microfluidic Devices Used in Low-Resource Settings" *Lab Chip*, **2016**, *16*, 4142-4151.
2. Apparatus Comprising Magnetically Actuated Valves and Uses Thereof. Edwards, T.L, Harper, J.C. US Patent: US 9,389,231 B2; Jul. 12, **2016**.
3. Harper, J. C., Carson, B. D., Bachand, G. D., Arndt, W. D., Finley, M. R., Brinker, C. J., Edwards, T. L. "Laser Machined Plastic Laminates: Towards Portable Diagnostic Devices for Use in Low Resource Environments" *Electroanalysis* **2015**, *27*, 2503-2512.
4. Amplification of Biological Targets via On-Chip Culture for Biosensing. Harper, J. C., Edwards, T. L., Carson, B. D., Finley, M. R., Arndt, W. D. Patent application (14/157,378) filed Jan 16, **2014**.

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