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PORTABLE DIAGNOSTIC DEVICE FOR THE DETECTION OF *BACILLUS ANTHRACIS* IN ULTRA-LOW RESOURCE ENVIRONMENTS

Jason C. Harper, Melissa Finley, Bryan Carson, Thayne Edwards,
George Bachand, Bill Arndt, and Julie Lovchik**
*Sandia National Laboratories, USA
**University of New Mexico, USA

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

Anthrax outbreaks commonly occur in livestock. Between 2005 and 2012, over 3000 anthrax outbreaks were reported, with the vast majority in the Middle East, Africa, and Southeast Asia. This is likely an underestimation of the incidence and prevalence of the disease. In these, and other resource limited regions, *B. anthracis* is routinely isolated, propagated, and maintained in laboratories (often unsecured) by indigenous populations to diagnose the disease. This practice drastically increases laboratories' repositories of *B. anthracis* and escalates the risk that it can be stolen for nefarious purposes. Moreover, it enhances the capabilities of laboratory personnel to produce pure isolates. That knowledge could potentially be used for terrorism.

To address these risks, we have developed a credit-card sized device for detection of *B. anthracis* in ultra-low resource environments called BaD_x (*Bacillus anthracis* Diagnostics, Figure 1). BaD_x is a disposable plastic device that is low cost ($5-$7/assay), requires no power, instrumentation, or equipment to operate, and no refrigeration to maintain efficacy. It requires little training or skill to use, detecting *B. anthracis* in the field with an accuracy that rivals laboratory analysis. Further, the device "self-destructs" by sterilizing all contents upon assay completion.

From an engineering and biology standpoint, meeting all these requirements in a single device is extremely challenging. Yet, the beauty of this device is its simplicity. The self-contained, credit-card sized device employs micro-culture to amplify bacteria prior to lateral flow assay (LFA). Using this technique, we have shown positive detection of only 100 virulent *B. anthracis* spores (Ames strain). This is a 4-5 orders of magnitude improvement in detection limit over LFA alone, bringing the detection limit within a practical range for real-world samples. Self-decontamination following assay greatly minimizes the potential for malicious use of the bacterial sample and improves safety for the operator.

We also significantly improve sensor performance through dual-selectivity. Selective growth medium is used for on-device micro-culture, allowing *B. anthracis* to grow while preventing growth of most competing bacteria, including many closely-related members of the *Bacillus* genus. Selectivity from the growth medium, in addition to the LFA selectivity, provides for highly reliable biodetection in the field, rivaling the accuracy of laboratory analysis. Further, we have shown amplification and detection of *B. anthracis* spiked into real-world samples (office, environmental, and agricultural).

We are aware of no other device that meets all these stringent performance requirements.

While designed to detect *B. anthracis* in resource-limited environments, the applications of BaD_x are significantly broad. BaD_x is readily modified to detect other bacteria. Immediate applications include food-borne bacteria (*E. coli, Salmonella*) and bacteria of medical interest (*Staphylococcus, Streptococcus, MRSA*). This customizable platform can thus revolutionize monitoring of bacteria within hospitals, health clinics, and homes.

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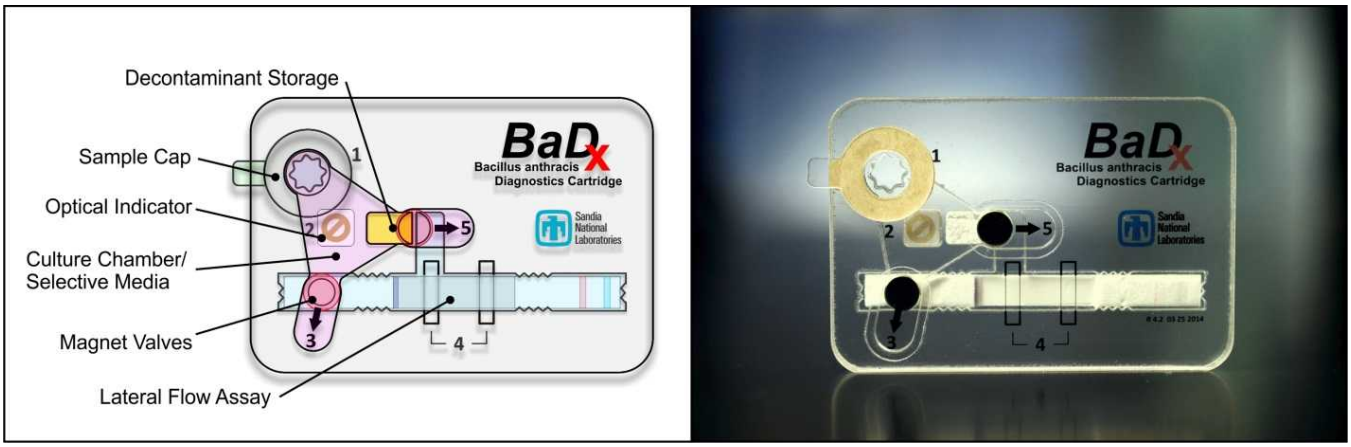

Figure 1. *Left;* Schematic of cartridge showing major components. *Right;* Photo of the fabricated cartridge with dimensions of 0.25 in. x 1.875 in. x 2.75 in.

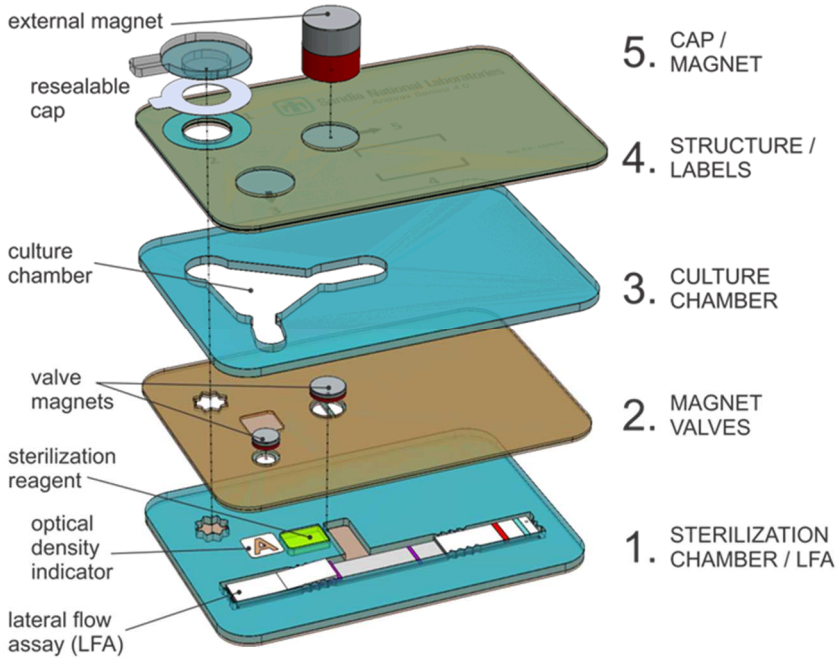

Figure 2. Exploded view of the various cartridge modules, also indicating the primary components of each module. BaD_x is fabricated using laser ablation of plastic laminates of various thicknesses joined together by adhesive on one or both sides of each laminate. The primary materials used in its construction are poly-(methyl methacrylate) (PMMA or acrylic) of various thicknesses, and thin layers of acrylic-based adhesive. Other materials used are high-strength neodymium magnets, printed paper, coated paper, and a lateral-flow assay.

REFERENCES:

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