PORTABLE DIAGNOSTIC DEVICE FOR THE DETECTION OF BACTERIA IN ULTRA-LOW RESOURCE ENVIRONMENTS

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ABSTRACT

Anthrax poses a significant threat to National Security as demonstrated by the terrorist attacks targeting the US Postal Service and Hart Building. Anthrax outbreaks commonly occur in livestock. Consequently, Bacillus anthracis is routinely isolated, propagated, and maintained to diagnose the disease. This practice increases laboratories’ repositories of the agent, escalating the risk that it can be stolen. We have developed BaDx (2014 R&D100 Awardee), a credit-card sized diagnostic device for use in ultra-low resource environments that is low cost, requires no power, instrumentation or equipment to operate, no cold chain, self-decontaminates post-assay, and is operable by individuals with little/no technical training.

KEYWORDS: Low Resource Environment Biodetection, On-chip Micro Culture, Lateral Flow Assay, Self-Decontamination, Anthrax, Bacillus anthracis

INTRODUCTION

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 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.

To mitigate these risks, we have developed a robust and portable diagnostic device for detection of bacteria in ultra-low resource environments. The disposable plastic device is low cost (<$5/assay), requires no power, neither instrumentation, nor equipment to operate, no refrigeration to maintain efficacy, can be operated by individuals with little to no technical training, and self-sterilizes upon completion of the assay.

EXPERIMENTAL

BaDx (Bacillus anthracis Diagnostics; pronounced Bad-X) is fabricated using laser ablation of laminates of various thicknesses. These laminates may have adhesive on one or both sides to join the various layers together. This fabrication method is useful for product development as changes can be quickly made, but is also conducive to large-scale manufacturing methods for commercialization. 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.

The cartridge consists of modules numbered 1 (bottom), to 5 (top). These modules, shown in Figure 1, represent a particular functionality of the product and also allow for simplified fabrication. Module 1 contains the two sensing features (optical density indicator and lateral-flow assay), and the dry sterilization reagent. Module 2 holds the valves (patent pending). Each valve is made from an orifice laser-ablated through the thickness of the PMMA layers, and sealed closed by a disc-shaped magnet. The magnet is held in place by the same adhesive that joins the PMMA layers together. This module has two...
valves, one for separating the culture chamber from the lateral-flow assay, and one for containing the sterilization agent. Module 3 comprises the micro-culture chamber for bacteria target amplification (patent pending). The valve magnets are also housed in this layer which restricts the movement of the magnets, while allowing the valves to open completely.

Module 4 is a mechanical layer as well as an instructional layer. It contains the simple directions for operation of the device and mechanical locators for positioning the external magnet for opening the valves. Module 5 is the re-sealable cap. It has a release liner that has an easy release side and a medium release side. Upon removal of the cap, the release liner stays on the cartridge to protect the adhesive during sample loading. The release liner is then removed to permanently join the adhesive layer of the cartridge to the adhesive layer of the cap.

Operation of BaDx is illustrated with numbered steps (1-5) corresponding with the cartridge labeling, as shown in Figure 1. First, the cap is removed and the sample is introduced into the culture chamber with water to reconstitute the freeze-dried selective growth media already stored in the culture chamber (step 1). The sample is allowed to grow for 8-18 hours until the visual indicator disappears due to the turbidity of the cultured cells (step 2). At that point an external magnet is placed over the valve in position 3 for a few seconds, and moved to the side opening the orifice and exposing the LFA to the sample (step 3). The schematic in Figure 3 demonstrates how the adhesive-based magnetic valving operates.

The sample is exposed to the LFA for approximately 15 to 20 minutes, at which time the LFA indicates the presence or absence or B. anthracis by a visual band (step 4). A control band also indicates whether or not the test is valid. Upon completion of the test, the external magnet is again used to open the valve in position 5 to release the dry decontamination reagent (step 5). Shaking the device distributes the reagent to all parts of the device, killing any biological materials.
RESULTS AND DISCUSSION

From an engineering and biology standpoint, meeting all the requirements for highly selective and sensitive detection of pathogenic detection in a single device is extremely challenging. Yet, the beauty of this invention is in its simplicity. Our self-contained, credit-card sized device employs on-chip microculture methods to amplify bacteria prior to lateral flow assay (LFA).

LFA technology (e.g. common home pregnancy tests) allows for highly specific antibody-based detection in a simple, user-friendly, one-step assay. However, a significant disadvantage of LFA is sensitivity. Detection limits range from 1 million to 10 million target particles. This sensitivity is not low enough to be practical in many cases where infectious doses are commonly 100-1000 bacteria cells.

To overcome the sensitivity limitation of LFA, BaDx incorporates a micro-culture chamber in the cartridge. This allows real-world samples containing as few as 100 bacteria cells to be amplified and detected in the field, rather than requiring it be sent to a lab.

Using this technique we have shown positive detection of only 100 spores of virulent B. anthracis (Ames strain, Figure 4, left). This is a 4-5 order of magnitude improvement in detection limit over LFA alone, and brings the device detection limit within a practical range for real-world samples.

We also significantly improve sensor performance through dual-selectivity. Selective growth medium (PLET++) is used for on-device micro-culture, allowing B. anthracis to grow while preventing growth of competing bacteria. Selectivity from the growth medium, in addition to the LFA selectivity, provides
Figure 4: Left, Photograph of the BaDx cartridge in operation with a positive control sample in the culture chamber which was introduced to the LFA through the magnetically actuated valve; Right, Detection of B. anthracis spores (sp) into real-world environmental and agricultural sample matrices.

for highly reliable biodetection in the field, rivaling the selectivity of laboratory analysis. Further, we have demonstrated amplification and detection of B. anthracis spiked into real-world samples (office, environmental, and agricultural), as shown in Figure 4 (right).

CONCLUSION

BaDx, a credit-card sized diagnostic device for use in ultra-low resource environments, is low cost, requires no power, instrumentation or equipment to operate, no cold chain, self-decontaminates post-assay, and is operable by individuals with little/no technical training. 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 BaDx are significantly broad. BaDx 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.

ACKNOWLEDGEMENTS

We thank Amada Carroll-Portillo, Jaclyn Murton, and Bryce Ricken for their invaluable assistance, and the Sandia Laboratory Directed Research and Development (LDRD) Program for funding. Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy’s National Nuclear Security Administration under contract DE-AC04-94AL85000.

REFERENCES


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