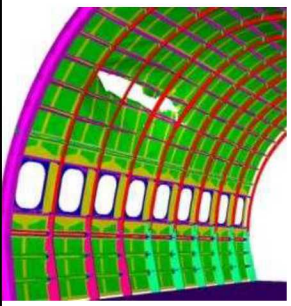
SAND2019-7257C



A "Universal" Bio-Sample Stabilization & Preservation Medium: Simple, Low-Cost Environmental Sample Collection and Storage

Robert K. Johnston, Jason C. Harper, Marilynn Larson, C. Jeffrey Brinker

Bioenergy & Biodefense Technologies, Sandia National Laboratories

National Strategic Research Institute/Univ. of Nebraska Medical Center

Chemical and Nuclear Engineering, University of New Mexico

Biodefense World Summit

Bethesda, MD

June 19, 2019

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Far-Forward Military Need for Bio-Sample Stabilization

Photo curtesy of militarysystems-tech.com

- Rapid in-field detectors are capable of providing preliminary analysis of biosamples
- Subsequent testing for validation or forensic analysis may be necessary
- Safe and secure collection and stabilization of biological samples would allow for:
  - ✓ Accurate identification of the biological
  - ✓ Proper treatment received for warfighters exposed to a potential biothreat
  - ✓ Vaccines received for biothreats (if available) prior to deploying forces

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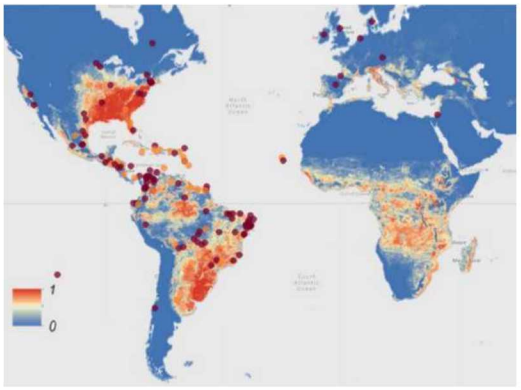
"Deadly diseases like Ebola, Marburg, and Anthrax are prevalent in Africa. These pathogens can be made into horrible weapons aimed at our troops, our friends and allies, and even the American public. This is a threat we cannot ignore." *-Senator Lugar,* stated during his 2010 trip to Africa

Ebola Outbreaks 2014

Zika Outbreaks 2015-2017



Anthrax Outbreaks 2011

Photos courtesy of Melissa Finley

Between 2005-2012 there were 3057 Anthrax outbreaks reported

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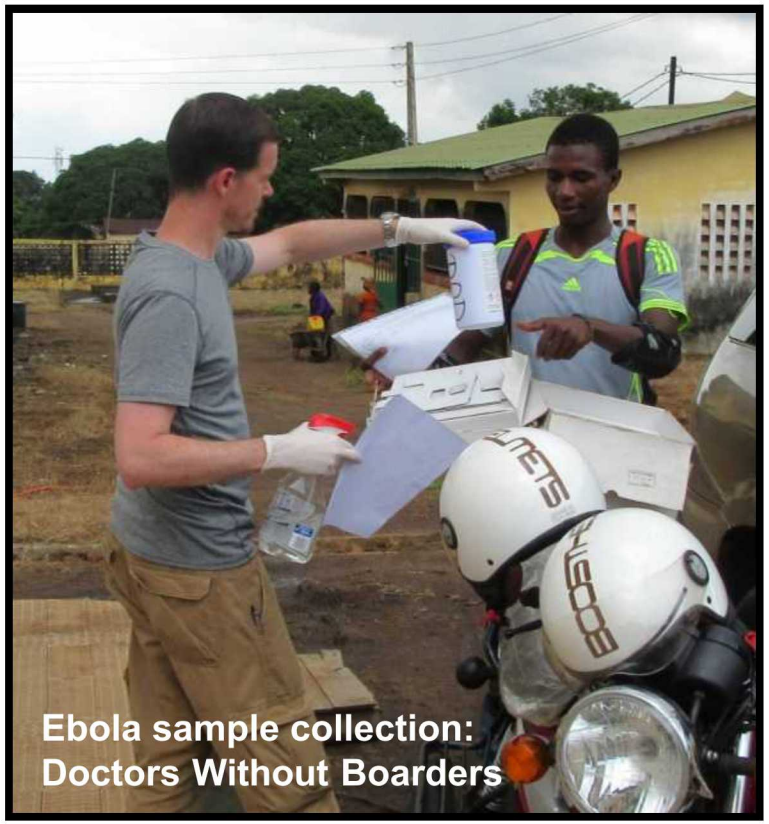
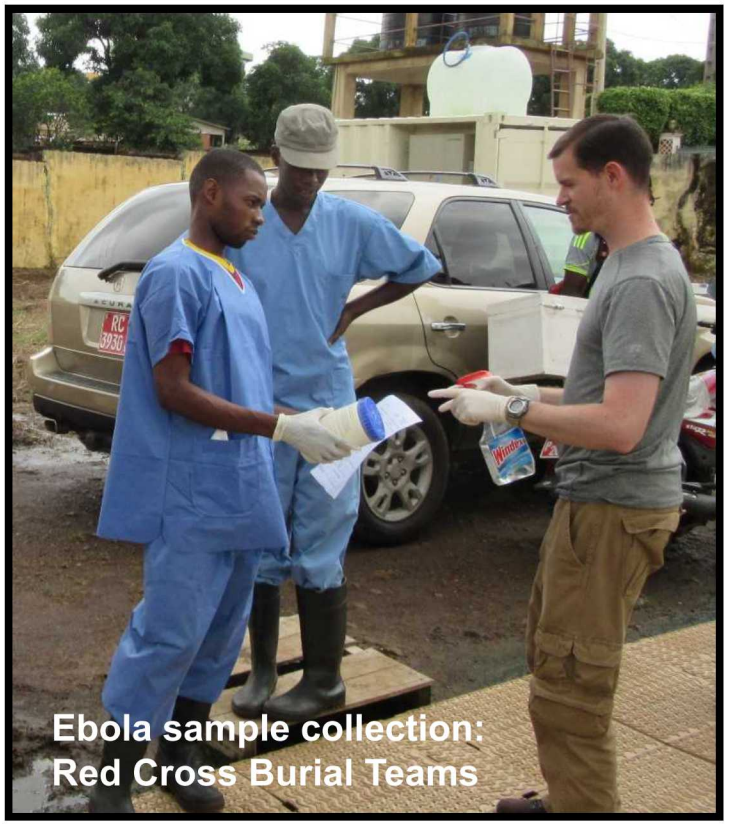
Ebola Outbreak Response Support
Conakry, Guinea



Ebola sample collection:
Doctors Without Boarders



Ebola sample collection:
Red Cross Burial Teams

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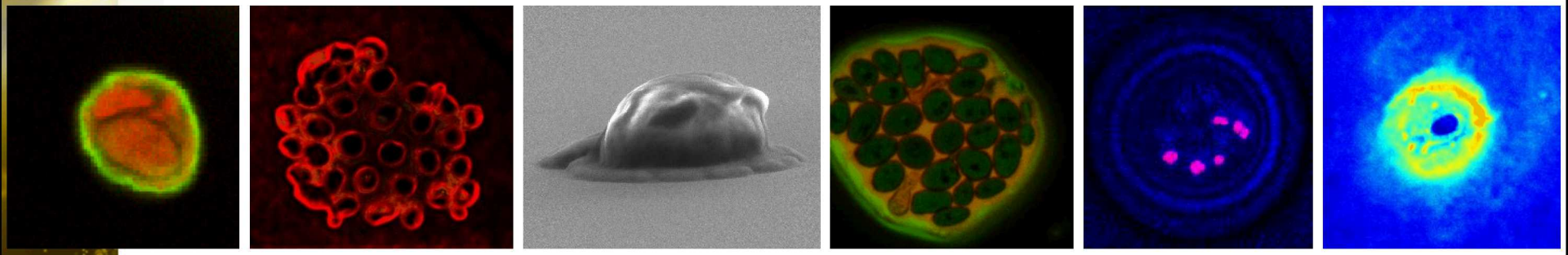
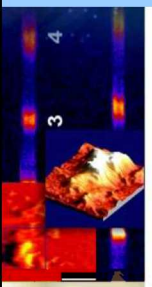
Encapsulation of Living Cells



Development of Living Biocomposites – Harness unique properties innate to biomolecules and living cells via 3D immobilization within matrices that preserve cellular behavior and accessibility to cells under *ex-vivo* conditions; Provide a biocompatible interface between immobilized cells and the macro world



Encapsulation of living cells in silica matrices has attracted considerable attention as these materials are *mechanically stable, chemically and biologically inert, easily processed at room temperature, retain water with negligible swelling, resist microbial attack, and can be tailored to provide desired porosity and other material and chemical properties.*†

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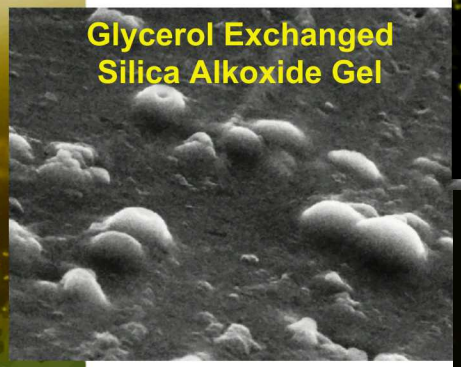
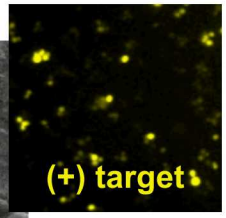
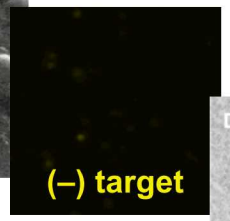
† Avnir *et al. J. Mater. Chem.* **2006**, *16*, 1013;
Nassif *et al. J. Mater. Chem.* **2003**, *13*, 203.

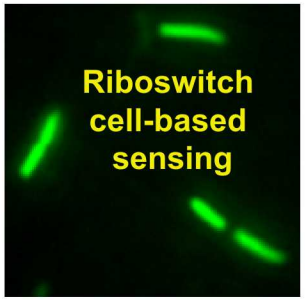
Technical Approach: Biocompatible 3D Silica Immobilization Strategies



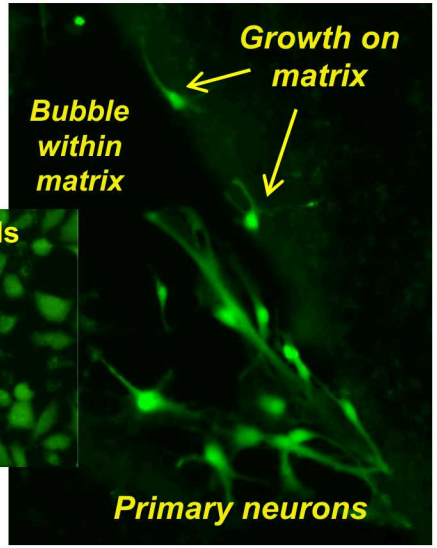


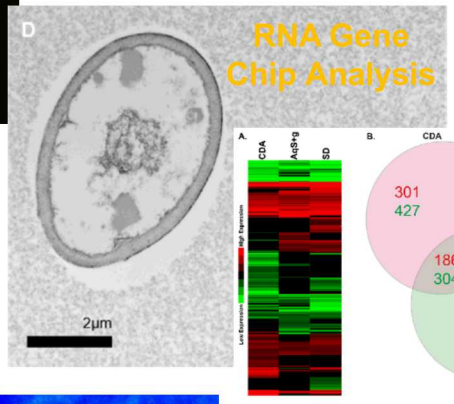


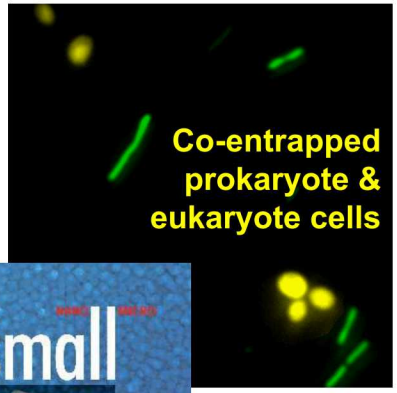
Harper *et al.*
Chem. Mater.
2011



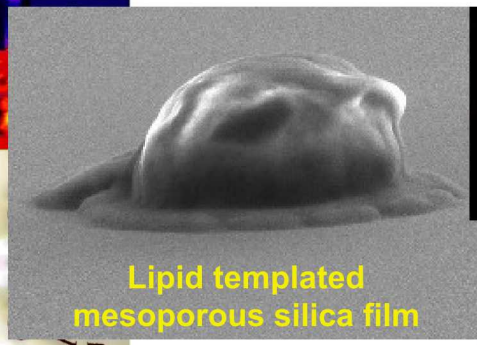
Savage *et al.*
ACS Biomater.
Sci. Eng. 2015

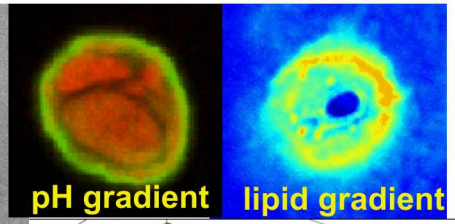


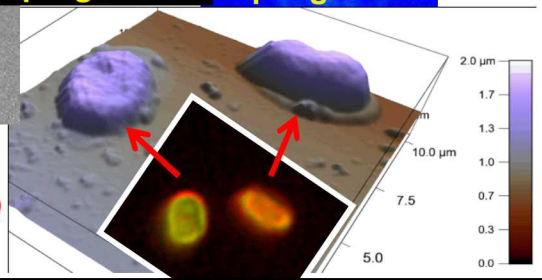




Fazal *et al.*
ACS Nano
2017







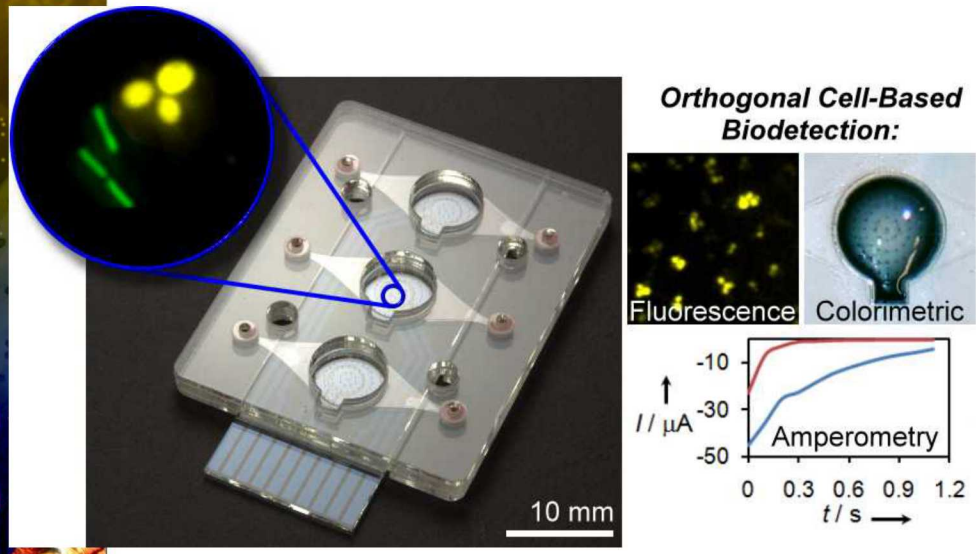
Harper *et al.*
ACS Nano 2010

Harper *et al.*
Small 2012

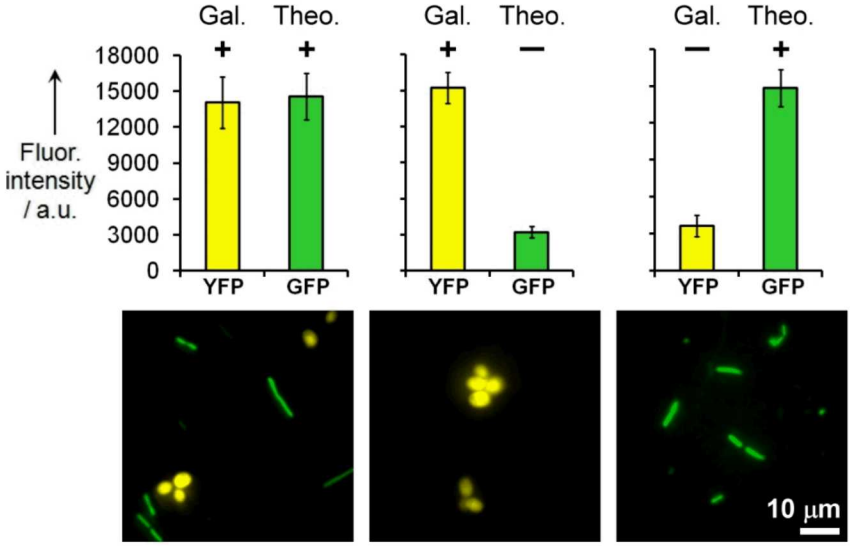
Orthogonal
fluorescence,
colorimetric, and
electrochemical
sensing

First Simultaneous Fluorescent, Electrochemical, & Colorimetric Detection via Silica Stabilized Cellular Communities

Orthogonal Fluorescent, Electrochemical, and Colorimetric Detection



Both Eukaryote and Prokaryote Cells Remain Viable and Responsive



→ *Multiple signals provide complementary data, increasing confidence in output*

→ *Orthogonality enabled by integrating multiple cell lines*

→ *First co-encapsulation of eukaryote and prokaryote cells*

→ *Enabled multi-analyte biodetection*

Harper et al. *Small.* **2012**, *8*, 2743

With Harbaugh, Kelley-Loughnane, and Stone, AFRL

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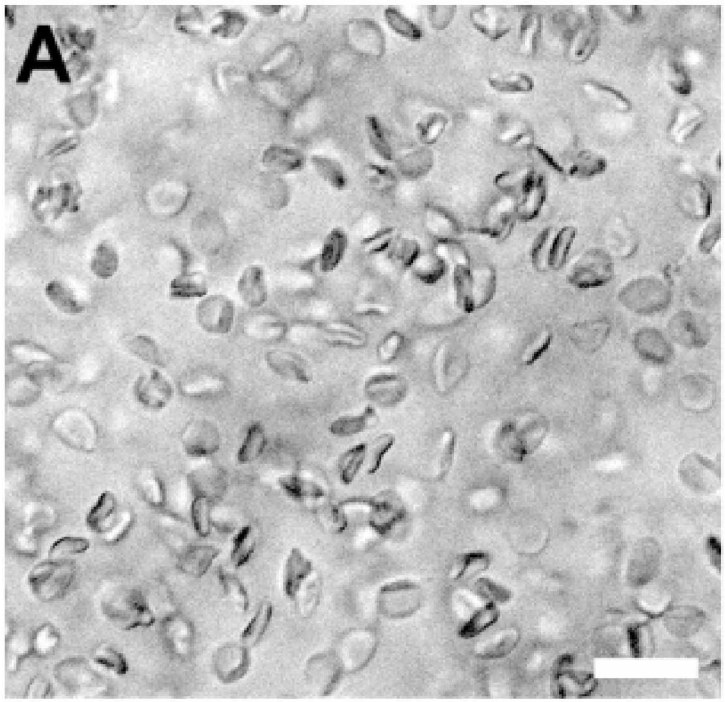
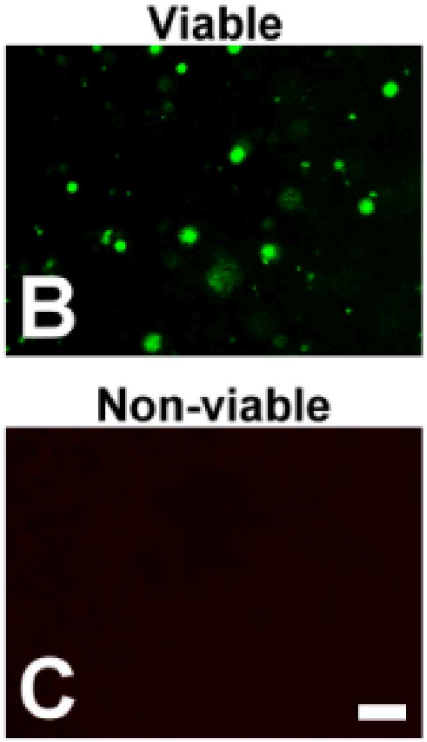
First Stabilization of Human Whole Blood in a Silica Gel Monolith

Human Whole Blood Stabilized in a Buffered Aqueous Silicate (AqS) based Inorganic Matrix

Photo of human whole blood stabilized in a AqS-based gel monolith



Bright field (black & white) image of red and white blood cells within a AqS-based matrix thin film. Scale = 20 μm.



Fluorescent microscopy images of whole blood within a AqS-based matrix thin film. Scale = 50 μm.

87% of white blood cells survive encapsulation and remain viable

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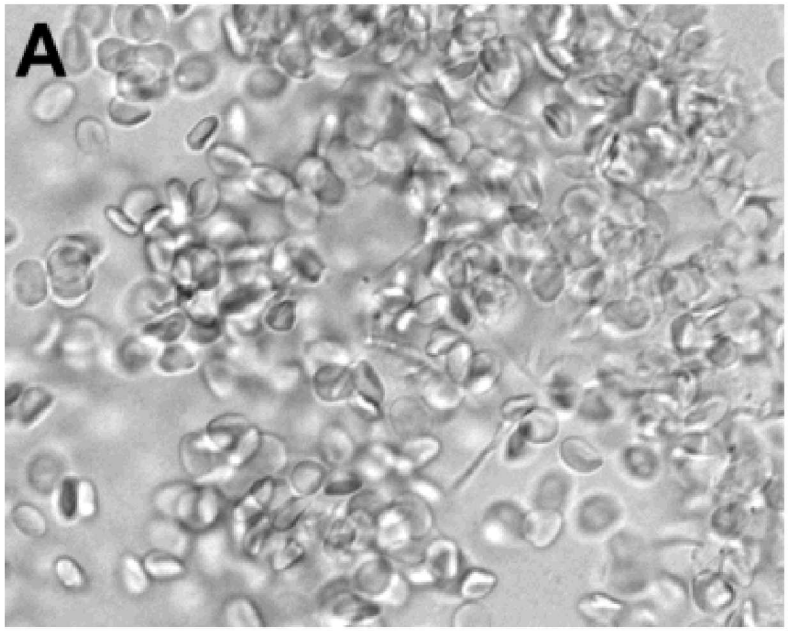
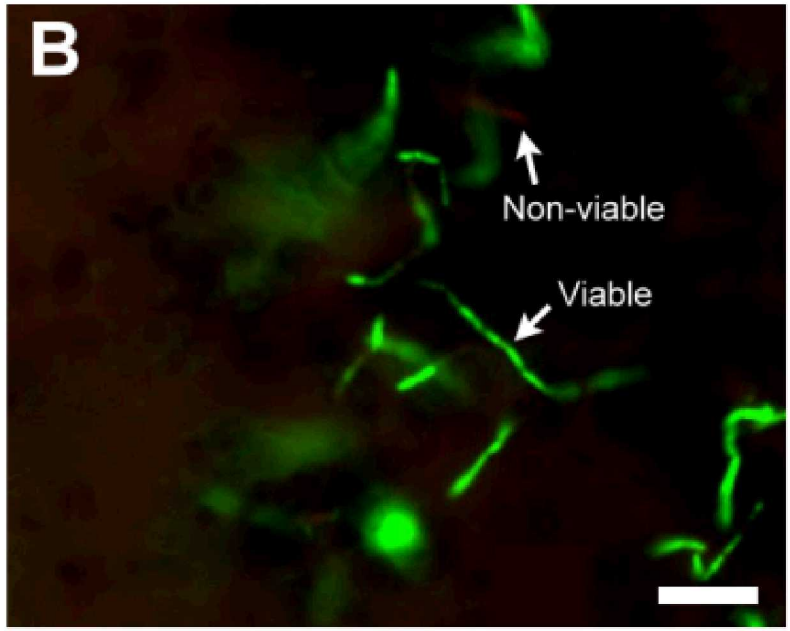
First Co-Stabilization of Human Whole Blood & Bacteria Pathogen in Silica Matrix

Human Whole Blood and *Bacillus anthracis* (Sterne) Stabilized in a Buffered Aqueous Silicate (AqS) based Inorganic Matrix



Bright field (black & white) image of human whole blood with *B. anthracis* (vegetative) stabilized within a AqS-based matrix thin film.



Merged fluorescence microscopy images of vital dye stained human whole blood and *B. anthracis.* Green stained bacteria and white blood cells are viable; red stained are non-viable. Scale = 20 μm.

92% of *B. anthracis* cells survive encapsulation and remain viable

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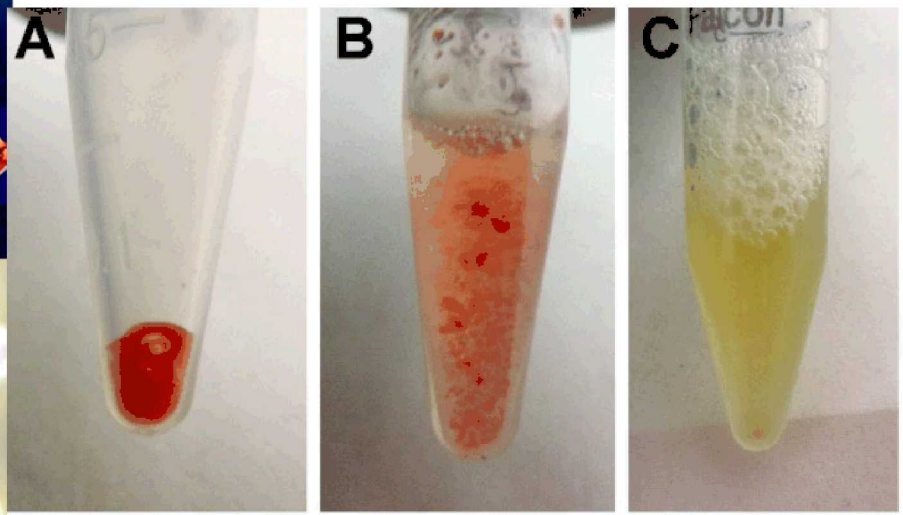
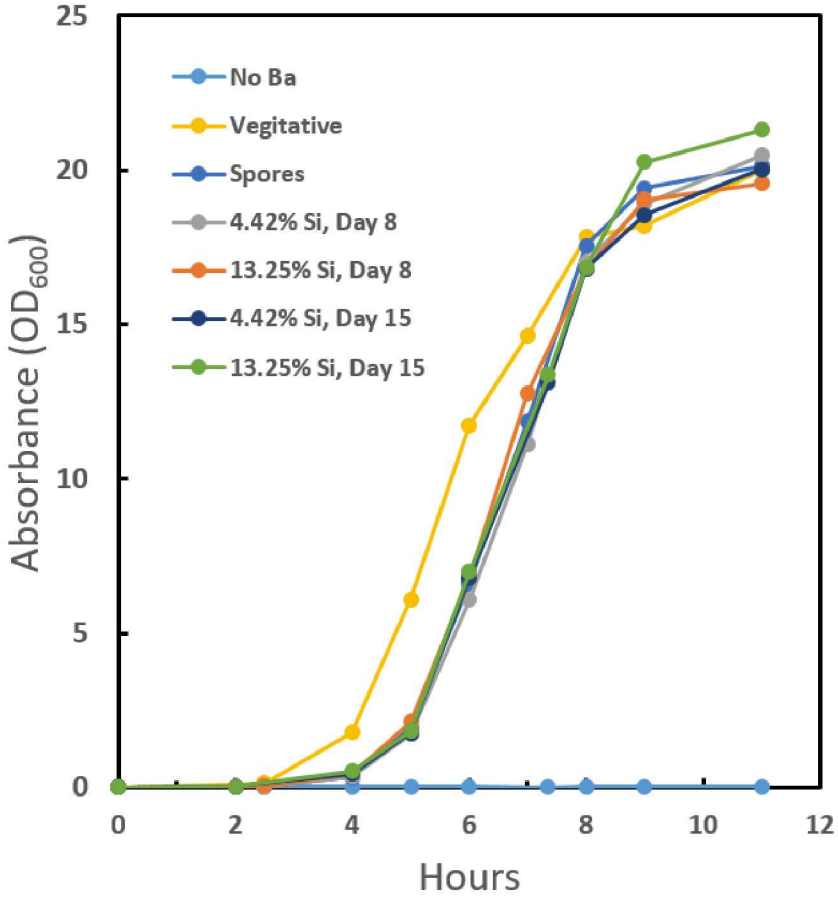
Extraction of Viable and Culturable *B. anthracis* from an Inorganic Silica Matrix

- Human whole blood spiked with *B. anthracis,* Sterne (vegetative), stabilized within an AqS-based silica matrix monolith
- Stored under ambient conditions for 3 days (22 C)
- Sample extracted w/ spatula, vortexed, resuspended in Heart Infusion (HI) media



Growth Curves Following Extraction of Stabilize Bio-Samples



Inoculated in 50 mL HI, 37 C incubation, 200 rpm

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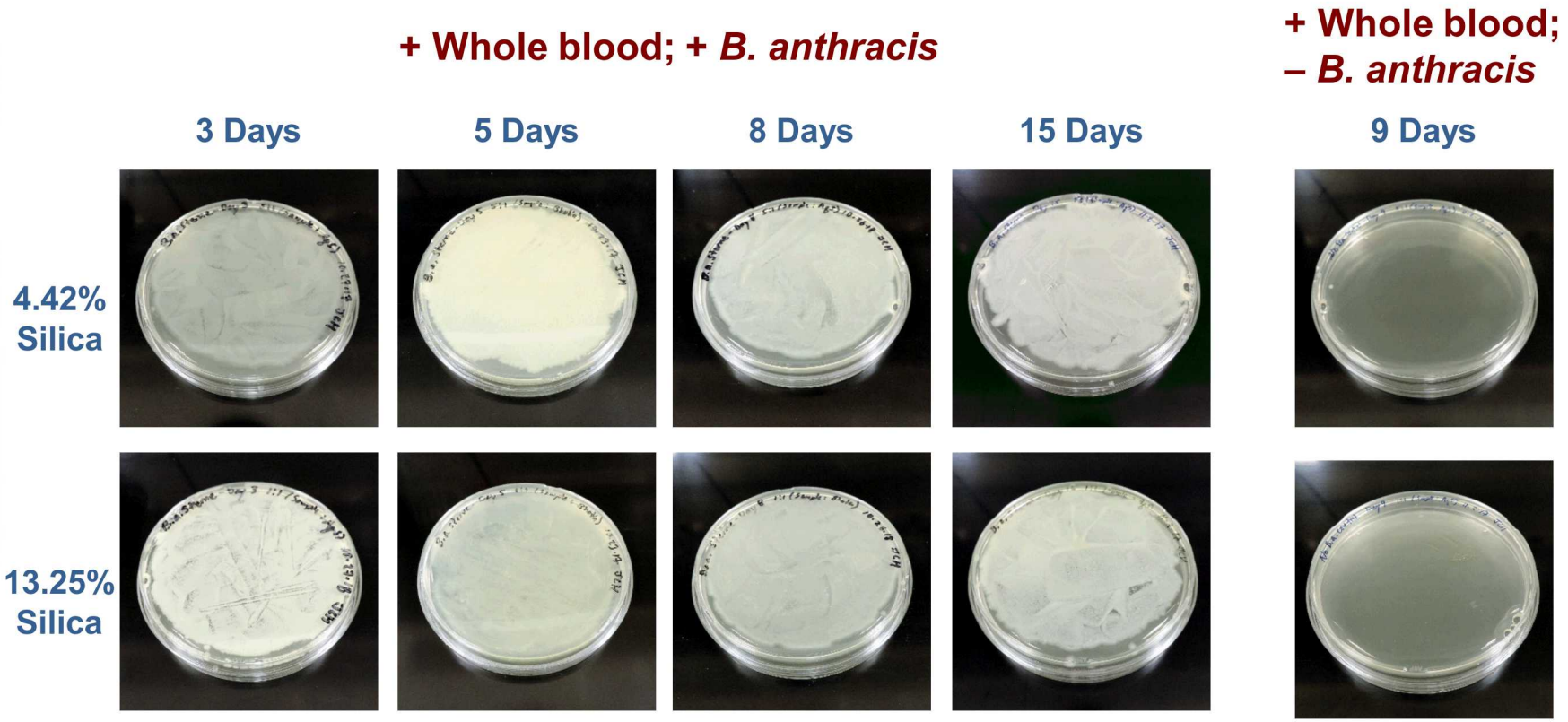
Culture on Solid Medium of *B. anthracis* Extracted from AqS-based Matrices

- Stabilized samples extracted with spatula, vortexed in 150 μL of 1x PBS, pH 7. This solution was plated (100 μL) and incubated overnight at 37 C.



Heart Infusion (HI) agar plates

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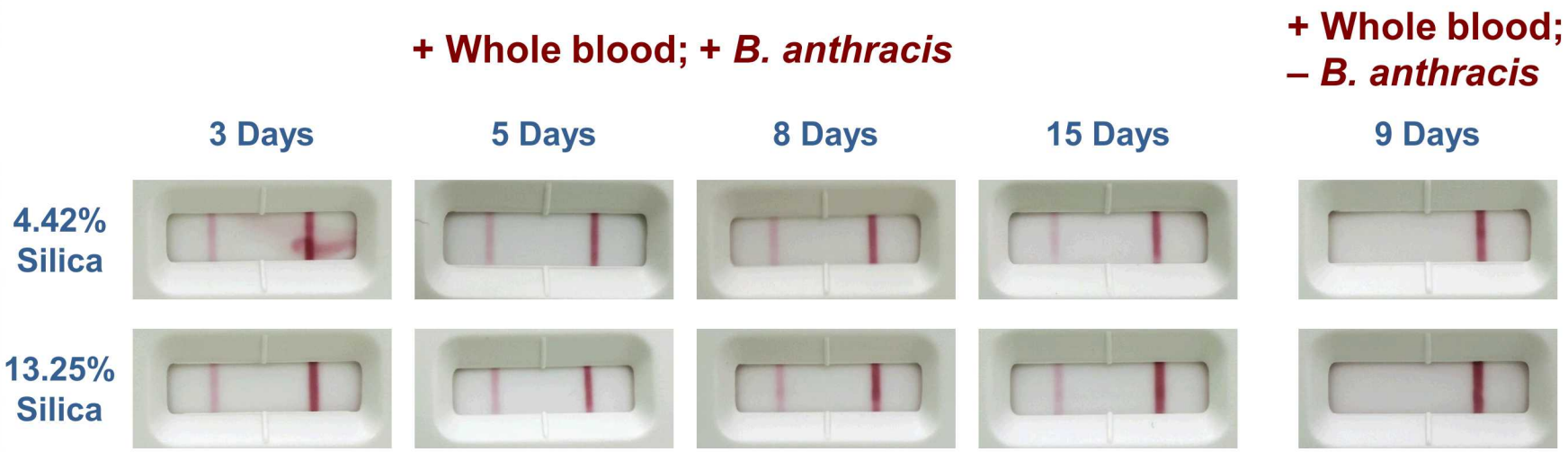
Use of Gold Standard Affinity Assay to Positively Identify Extracted *B. anthracis*

- Following overnight incubation on HI-agar, a colony was picked from the plate, resuspended in colony isolation buffer, and loaded onto a lateral flow assay.



RedLine Alert *Bacillus anthracis* lateral flow assay, TetraCore

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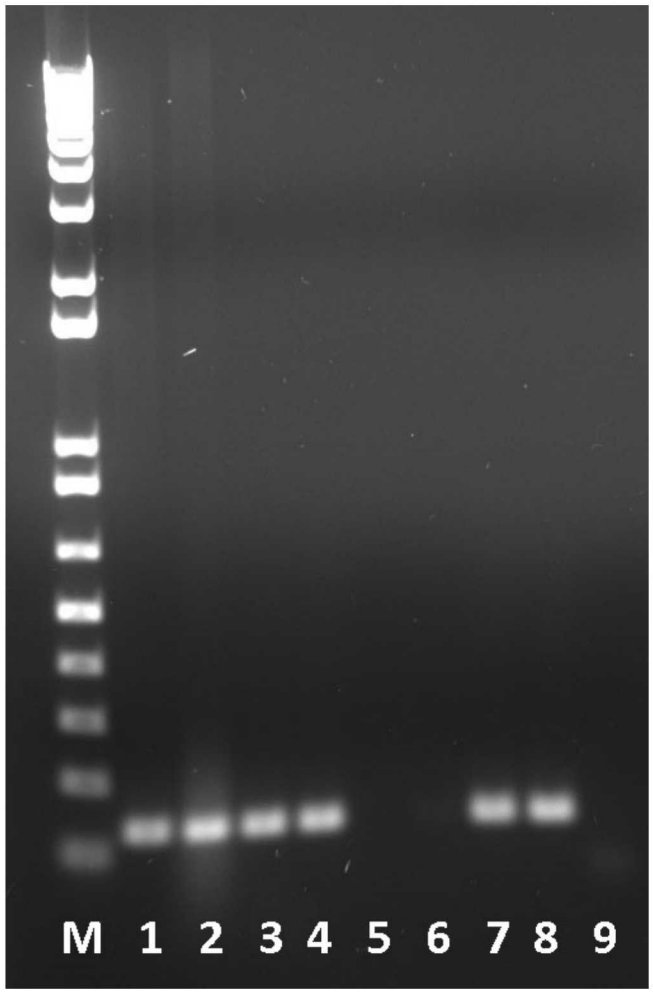
Stabilized Samples Sent from Albuquerque to Omaha; PCR of Extracted Sample

PCR Products from Extracted Sample Identify Presence of *B. anthracis*



- Stabilized samples shipped from Sandia Labs to Univ. of Nebraska Medical Center without ice/dry ice; stored at ambient for 3+ weeks

M: Marker, 1kD DNA ladder

1: *B. anthracis* genomic DNA (+ control)

2: DNA extracted from *B. anthracis* culture (+ control)

3: Si Stabilized *+ B. anthracis*, cultured day 18, pelleted and frozen, DNA extracted on day 25; 4.42% Si

4: Si Stabilized *+ B. anthracis*, cultured day 18, pelleted and frozen, DNA extracted on day 25; 13.25% Si

5: Si Stabilized *– B. anthracis*, cultured day 18, pelleted and frozen, DNA extracted on day 25; 4.42% Si

6: Si Stabilized *– B. anthracis*, cultured day 18, pelleted and frozen, DNA extracted on day 25; 13.25% Si

7: Si Stabilized *+ B. anthracis*, DNA extracted directly on day 25; 4.42% Si

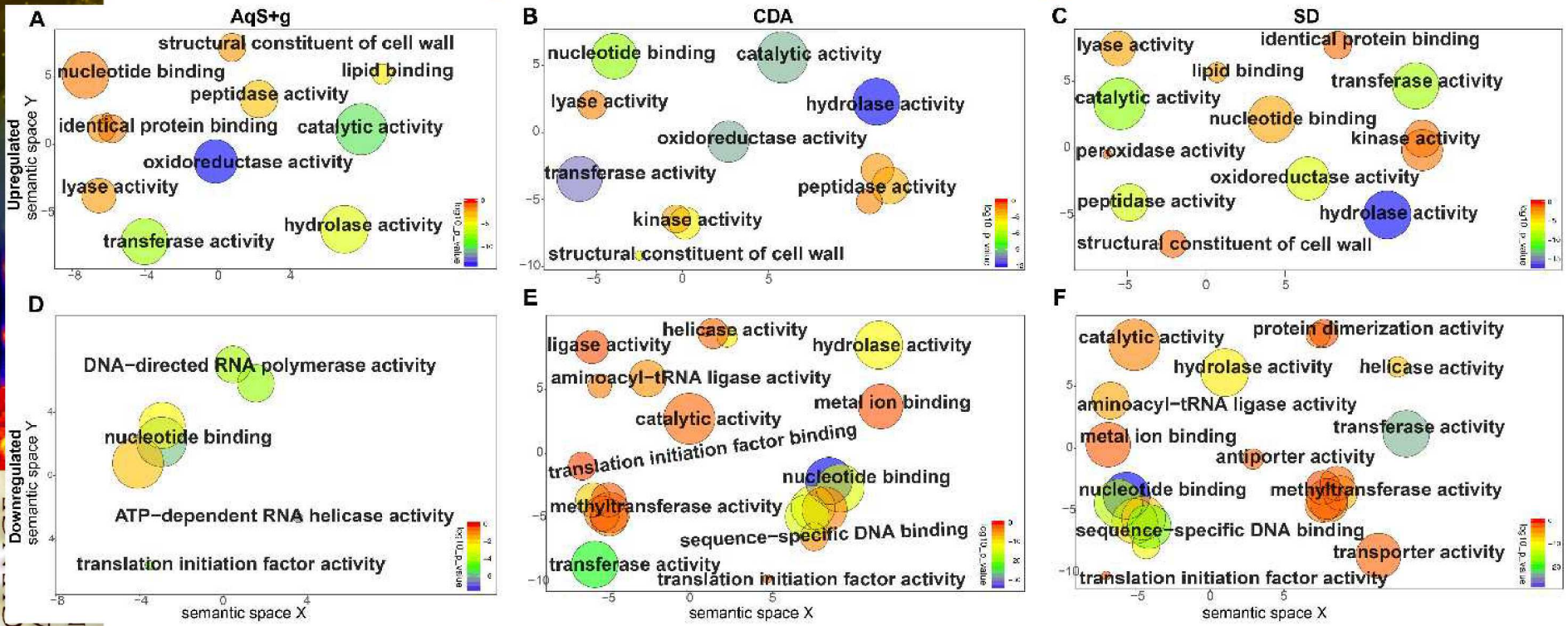
8: Si Stabilized *+ B. anthracis*, DNA extracted directly on day 25; 13.25% Si

9: No template (– control)

Use of RNA Gene Chip Assay to Probe Metabolic State of Si Matrix Entrapped Cells

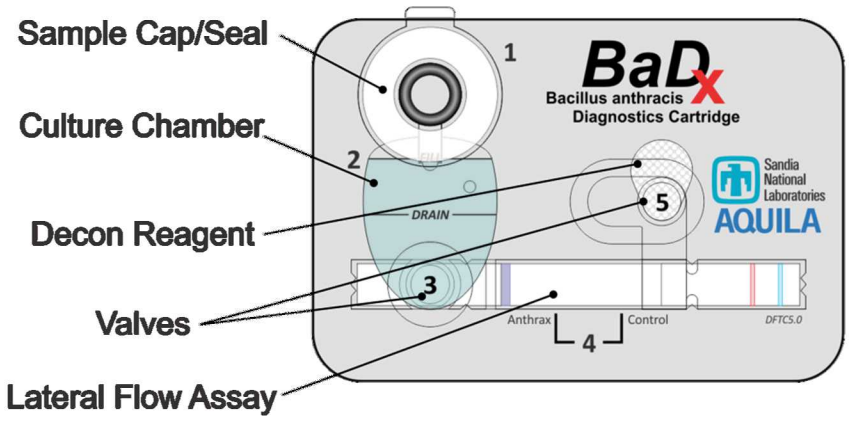
- *S. cerevisiae* cells stabilized in 3 differing silica matrices
- Cells extracted and RNA gene chip assay performed (Affymetrix) to measure gene expression profiles

Biological Processes Gene Ontology Terms



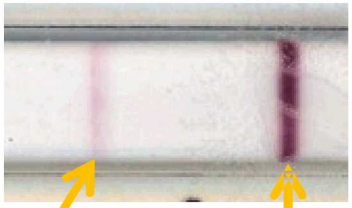
Frazal, Harper et al. *ACS Nano* **2017**, *11*, 3560

B. anthracis Diagnostics (BaD_X) Cartridge



Self-contained, credit card-sized "Laboratory in a Pocket" for bacterial detection, containment, and destruction

Ames (BSL 3)
100 spore inoculum



- ✓ Field deployable
  - Credit card sized
  - Rugged/robust
- ✓ No power to operate
- ✓ No instrumentation or equipment to operate/read
- ✓ *Detection as low as 1 viable spore demonstrated*
- ✓ Self-sterilizing upon assay completion
- ✓ Operable by individuals with little to no technical training

Commercially available – Aquila Technologies: $98.99

R&D 100 2014 Winner
2015 FLC National Awards Excellence in Technology Transfer
Best Tech of the Year Popular Science
TechConnect Innovation Award

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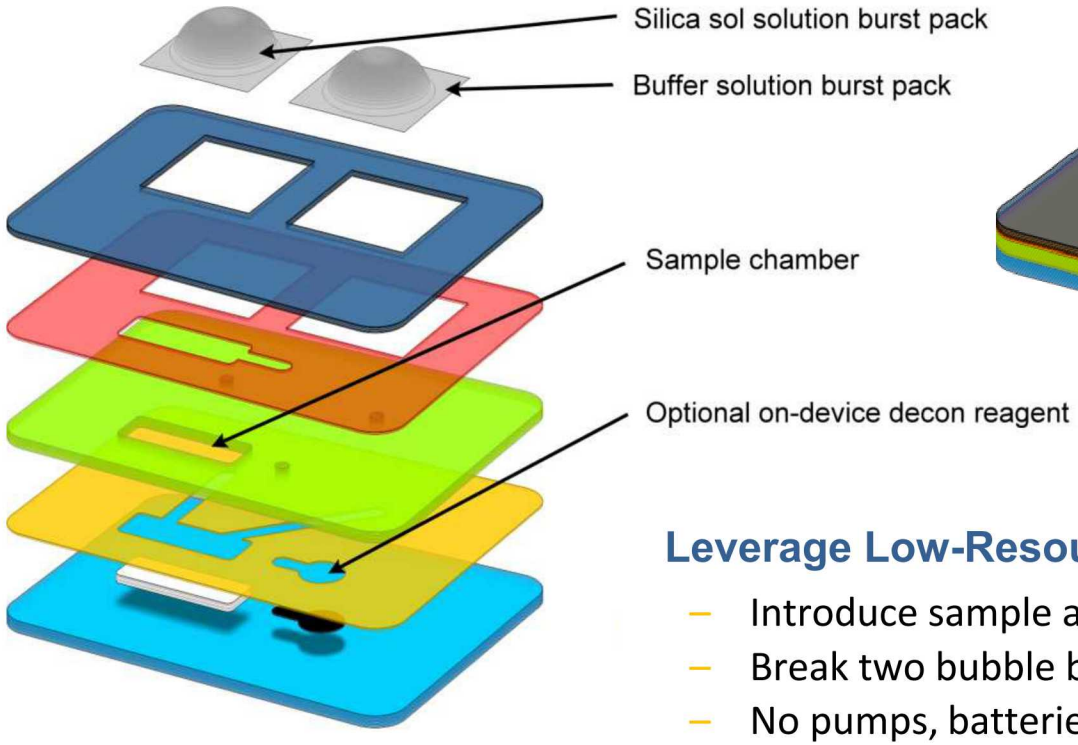
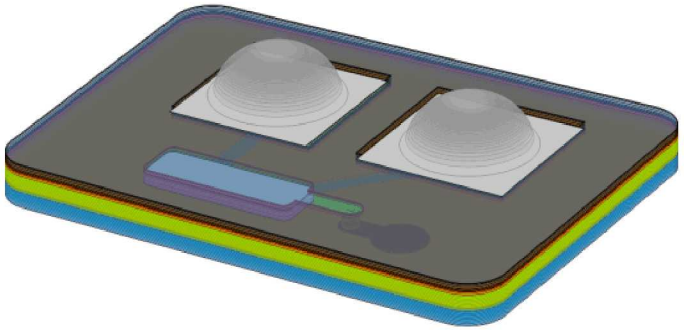
Use of Bio-Sample Stabilization Chemistry in Far-Forward or Low-Resource Settings

Proof-of-Concept Universal Bio-Sample Stabilization Device





This device could serve as a back-end to current far-forward detectors, or as a stand-alone unit

Leverage Low-Resource Pathogen Detection Tech

- Introduce sample and seal device
- Break two bubble burst packs and shake device
- No pumps, batteries, or other external equipment required to operate
- Low cost: < $2 chemistry; < $5 BOM
- High stability - chemistry stable for several years w/o cold chain

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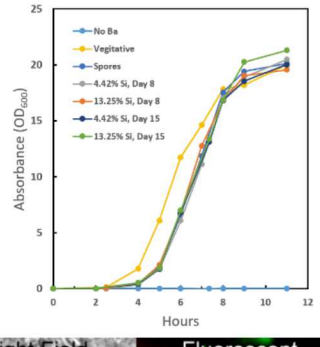
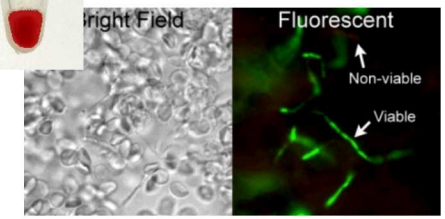
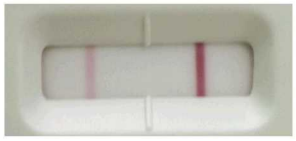
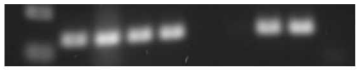
Summary & Future Work

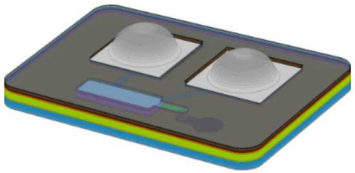




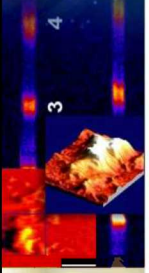
- ***First stabilization of human whole blood in an inorganic silica matrix***
  - 87% of white blood cells remain viable post-encapsulation
- ***First co-stabilization of human whole blood and pathogen (B. anthracis) in an inorganic silica matrix***
  - 92% of *B. anthracis* remain viable post-encapsulation
- ***Extracted viable and culturable samples following storage under ambient conditions for 2+ weeks***
  - Positive culture on liquid & solid medium
  - Positive identification of pathogen by affinity assay (LFA)
  - Positive ID of pathogen via PCR after shipping from Albuquerque to Omaha w/o cold chain, 25 days storage
  - Extracted nucleic acid used with standard RNA gene chip







- Extend to other select agent pathogens:
  - RNA virus, DNA virus, other bacteria
- Test in other complex matrices:
  - Oral swab, mucosal swab, urine, fecal matter, tissue
- Develop hand-held, low cost device for stabilizing bio-samples



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Dr. Nancy Kelley-Loughnane
Dr. Morley Stone

University of New Mexico

Prof. Jeff Brinker

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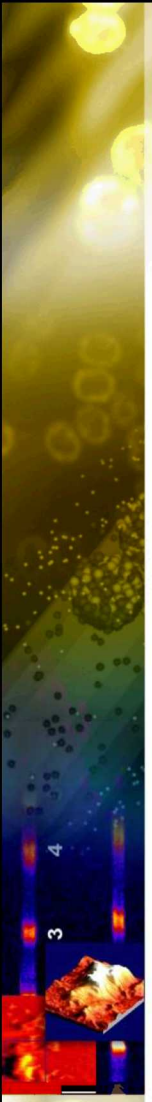
U.S. Department of Energy



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