



# Development of a rapid nucleic acid sample preparation device for point-of-care diagnostics

Vandevanter PE<sup>1</sup>, Salazar J<sup>1</sup>, Erwin B<sup>1</sup>, Ferguson T<sup>1</sup>, Doebler R<sup>2</sup>, Irvine B<sup>2</sup>, Morgan P<sup>2</sup>, Perez M<sup>3</sup>, Voelkerding K<sup>3,4</sup>, Sterling J<sup>1</sup>, Niemz A<sup>1</sup>.

<sup>1</sup>Keck Graduate Institute, Claremont, CA, <sup>2</sup>Claremont BioSolutions, Upland, CA, <sup>3</sup>ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, and

<sup>4</sup>University of Utah, Department of Pathology, Salt Lake City, UT.



## Introduction

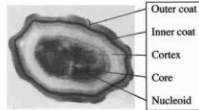
### Overall goal:

- Develop a miniaturized, rapid, inexpensive, battery powered, and handheld sample preparation device for lysis of tough walled pathogens and solid phase extraction of nucleic acids in point of care (POC) settings
- Will eventually be combined with isothermal DNA amplification and lateral flow based detection into a fully integrated cartridge for nucleic acid testing to enable rapid pathogen diagnosis at the POC

## Examples of Tough-Walled Pathogens

### Bacillus anthracis spores:

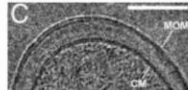
- Protective coat consisting of multilayered protein shell
- B. subtilis* spores are a BSL1 surrogate for *B. anthracis* spores



Electron micrograph of a *B. subtilis* spore  
Microbiol. Mol. Biol. Rev. 2000, 64, 548-572

### Mycobacterium tuberculosis:

- Thick waxy cell envelope with low permeability
- M. bovis BCG* is a BSL2 surrogate for *M. tuberculosis*



Cryo-electron micrograph of *M. bovis BCG*; PNAS, 2008, 105, 3963-3967

## Methods to Lyse Tough-Walled Pathogens

- Chemical lysis: often ineffective
- Heat lysis: introduces additional heating step, can damage nucleic acids
- Mechanical lysis (bead-beating or sonication): most effective method, but typically requires a benchtop instrument



Cepheid GeneXpert™ - incorporates ultrasonic lysis



BioSpec Mini-Beadbeater™

### ClaremontBio blender:

- Small, disposable, inexpensive, battery operated sample preparation device
- Developed at Keck Graduate Institute of Applied Life Sciences (KGI) and now produced at Claremont Biosolutions (ClaremontBio)



- Two versions: OmniLyse™ (Cell lysis only) and PureLyse™ (Cell Lysis and solid phase nucleic acid extraction)

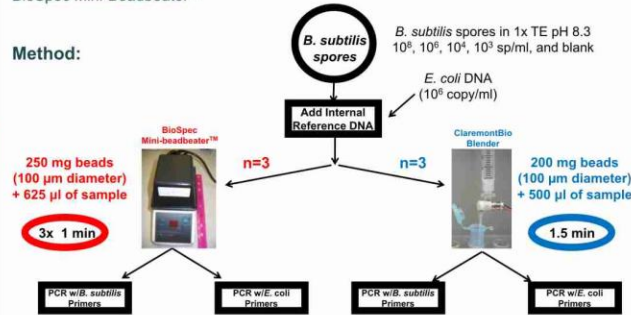
- Enables rapid lysis (< 2 min) and solid phase nucleic acid extraction (5-10 min)
- Injection molded body with miniature motor and vane, contains pre-treated beads
- Enables flow-through processing of large sample volumes, inlet compatible with syringe or pipette tip

## Lysis of *B. subtilis* spores – Comparative Performance

### Purpose:

Compare relative lysis efficacy of *B. subtilis* spores using ClaremontBio blender and BioSpec Mini-Beadbeater™

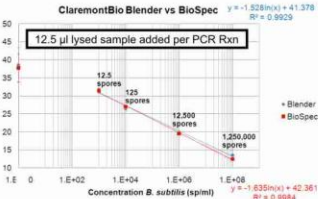
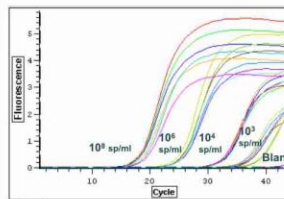
### Method:



- Lysed samples analyzed by real-time PCR using primers for *B. subtilis* or *E. coli*
- Comparison of lysis efficiency based on comparison of Ct values
- Incorporation of internal control to account for secondary effects

### Results:

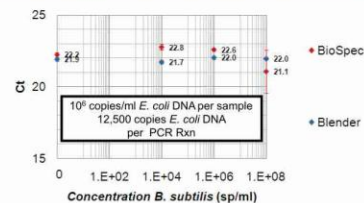
#### *B. subtilis* PCR (Sample)



- B. subtilis* ct values obtained for entire concentration range comparable for both devices

#### *E. coli* PCR (Internal Control)

- Ct values of internal control (*E. coli* DNA) are comparable for both devices



### Conclusions:

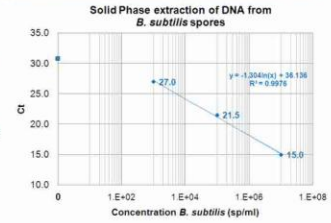
- B. subtilis* spore lysis efficacy using the miniaturized ClaremontBio blender is comparable to the benchtop BioSpec Mini-Beadbeater™
- Experiment not confounded by secondary effects (e.g. DNA degradation, PCR inhibition, DNA adsorption to beads etc)

## Sample preparation using the PureLyse™ System

- ClaremontBio blender enables solid phase DNA extraction from *B. subtilis* spores, *E. coli*, and from Herpes Simplex Virus (swabs of viral cell culture)
- Simplified two step protocol: Lysis/capture, followed by elution, no chaotropic salts

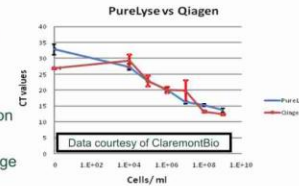
### *B. subtilis* spores

- Proof of concept for lysis and solid phase DNA extraction
- Log-linear dose response of Ct values versus starting concentration of bacterial spores



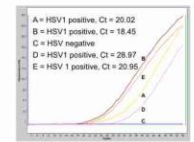
### *E. coli* cells

- Performance comparison: PureLyse™ blender and Qiagen DNeasy™ Kit for gram-negative bacterial cells
- Ct values obtained for entire concentration range are comparable - both methods showed similar efficacy over dilution range



### HSV infected Human Cell Line

- DNA extracted via PureLyse™ blender from swabbed shell vials infected with residual HSV positive clinical samples is readily PCR amplified, with Ct values indicative of 10<sup>7</sup> to 10<sup>5</sup> copies of HSV DNA



## Conclusions and Future Directions

### Conclusions

- ClaremontBio OmniLyse™ blender enables lysis of tough-walled pathogens with similar efficiency as industry standard benchtop bead beating systems
- ClaremontBio PureLyse™ blender enables solid phase DNA extraction from bacterial and viral pathogens

### Future Efforts

- Expand lysis efforts to other tough walled pathogens such as *M. tuberculosis*
- Refine method for solid phase extraction of nucleic acids
- Combine sample preparation, isothermal amplification, and lateral flow based detection into a fully integrated closed unit cartridge, addressing the need for simple, rapid, nucleic acid infectious disease diagnosis at the POC in low resource settings

## Acknowledgements

### Funding:

- NIH: NIAID (R01AI076247)
- DOD: Science, Mathematics And Research for Transformation (SMART) Scholarship
- Keck Graduate Institute