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

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Introduction

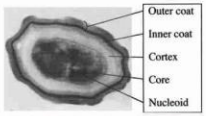
Overall goal:

- 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*:**

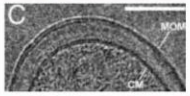
- Protective coat consisting of multilayered protein shell
- *B. subtilis* spores are a BSL1 surrogate for *B. anthracis* spores



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

- Appl. Environ. Microbiol., Jan. 2009, 414–418

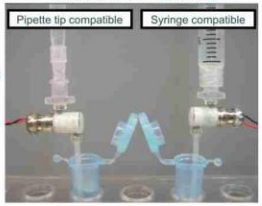
Cepheid GeneXpert™ - incorporates ultrasonic lysis

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)

ClaremontBio Blender



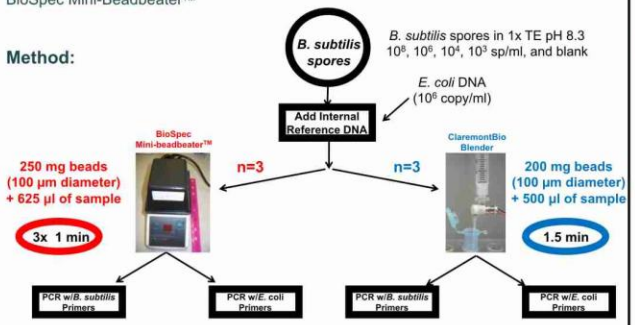
- Enables rapid lysis (< 2 min) and solid phase nucleic acid extraction (5-10 min)
- Injection molded body with miniature motor and vane, contains pre-treated beads
- Enables flow-through processing of large sample volumes, inlet compatible with syringe or pipette tip

Lysis of *B. subtilis* spores – Comparative Performance

Purpose:

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

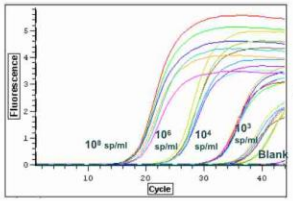
Method:

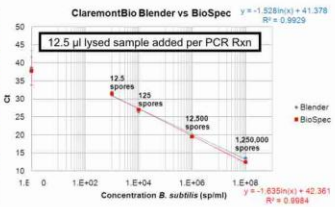


- Lysed samples analyzed by real-time PCR using primers for *B. subtilis* or *E. coli*
- Comparison of lysis efficiency based on comparison of Ct values
- Incorporation of internal control to account for secondary effects

Results:

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

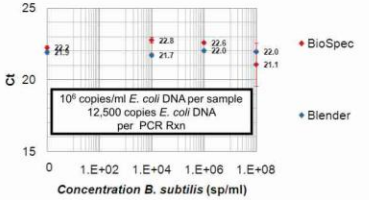




- *B. subtilis* ct values obtained for entire concentration range comparable for both devices

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

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



Conclusions:

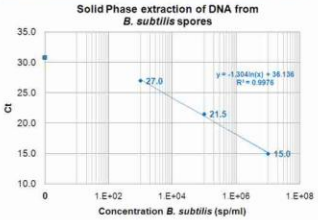
- *B.subtilis* spore lysis efficacy using the miniaturized ClaremontBio blender is comparable to the benchtop BioSpec Mini-Beadbeater™
- Experiment not confounded by secondary effects (e.g. DNA degradation, PCR inhibition, DNA adsorption to beads etc)

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

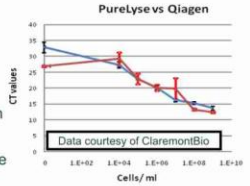
***B. subtilis* spores**

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



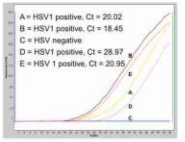
***E. coli* cells**

- 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 10^7 to 10^5 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

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