Highly Rapid Molecular Detection of *Burkholderia pseudomallei* in Soil

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Abstract

Recently thought to only be endemic to Southeast Asia and Northern Australia, *Burkholderia pseudomallei* is now predicted to be present in soil throughout almost all tropical regions of the world and is estimated to kill 89,000 people per year. Many of these regions have limited resources and adequate/timely detection methods for *B. pseudomallei* are unavailable. Rapid, accurate, and cost-effective detection of *B. pseudomallei* in soil is desperately needed to assess this emerging threat and prevent exposure. In this study, we applied a three minute nucleic acid preparation procedure consisting of two simple pipetting steps to detect *B. pseudomallei* in soil. Soil samples were spiked with *B. pseudomallei* and rapidly prepared for real-time PCR and loop-mediated isothermal amplification (LAMP) analyses using the ARCIS DNA prep kit. Three soil types were tested: Sassafras Sandy Loam (SSL), Richfield Clay Loam (RCL), and humus. The ARCIS kit was comparable to the DNeasy PowerSoil Kit which takes approximately two hours and requires a vortex, centrifuge, and refrigeration. The strong chelators present in the ARCIS kit were found to effectively neutralize humic acid, an amplification inhibitor found in organic matter, even in soil having an organic matter content close to 100%. The limit of detection (LOD) was estimated to be between 3.8–38 cells for SSL and LAMP. We plan to integrate lyophilized ARCIS reagents into a microfluidic cassette for automated sample preparation in a low-cost, field-deployable detection device. The goal is to be able to detect *B. pseudomallei* from raw soil samples within 30 minutes when combined with a highly sensitive and specific isothermal amplification assay.

Methods

Soil Sample/DNA Preparation

Soil samples were spiked with varying amounts of *B. pseudomallei* strain 82 (Δ*purM*) resuspended in 10 mM $CaCl_2$. DNA from the spiked soil samples was prepared using either the ARCIS Sample Prep Kit (ARCIS Biotechnology, Daresbury, UK) or the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany), following their protocols. The ARCIS procedure consists of two pipetting steps and a 1 minute incubation at room temperature. For the ARCIS prep, the soil was first liquefied with 10 mM $CaCl_2$ and the soil supernatant was used, while dry soil was used for the DNeasy PowerSoil prep. The DNeasy PowerSoil Kit is known to provide high purity DNA for downstream applications from a wide variety of soil types and was used for comparison.

Real-time PCR (RT-PCR)

RT-PCR assays were performed using *Burkholderia* Target 3 FastBlock Master Mix [Defense Biological Product Assurance Office (DBPAO), Frederick, MD], Platinum Taq DNA Polymerase (ThermoFisher, Waltham, MA), and varying concentrations of sample DNA. The *Burkholderia* Target 3 Positive Control (DBPAO) was used with each assay, along with a negative control (water). Additional positive controls consisting of ARCIS/PowerSoil-prepped DNA from *B. pseudomallei* culture (without soil) were used in each assay. The assays were run on an ABI7900HT Fast instrument.

Real-time LAMP (RT-LAMP)

RT-LAMP assays were performed using Bst 2.0 WarmStart DNA Polymerase [New England BioLabs (NEB), Ipswich, MA], 1X Isothermal Amplification Buffer (NEB), 10 mM dNTPs, 100 mM $MgSO_4$, six *B. pseudomallei*-specific LAMP primers (designed by Univ. of Florida), 5 mM Syto 9 Green Fluorescent Nucleic Acid dye (ThermoFisher), and varying concentrations of sample DNA. Positive controls consisting of ARCIS/PowerSoil-prepped DNA from *B. pseudomallei* culture (without soil) were used in each assay. The LAMP reactions were run at 70°C for 1 hr on the ABI7900HT Fast instrument.

Results

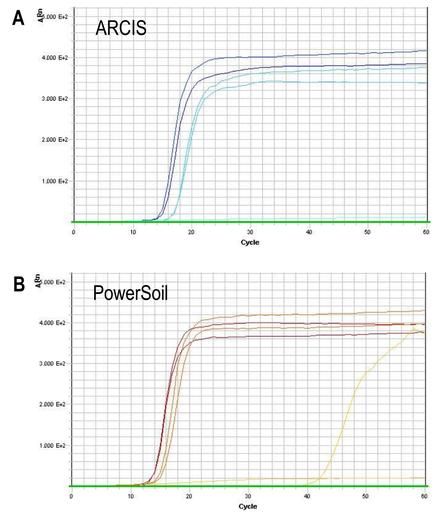

Fig. 1. ARCIS vs. PowerSoil in spiked SSL. DNA prepared from the ARCIS Kit (A) was compared to DNA prepared from the PowerSoil Kit (B) to determine suitability for RT-LAMP detection of *B. pseudomallei* spiked in SSL using tenfold dilutions.

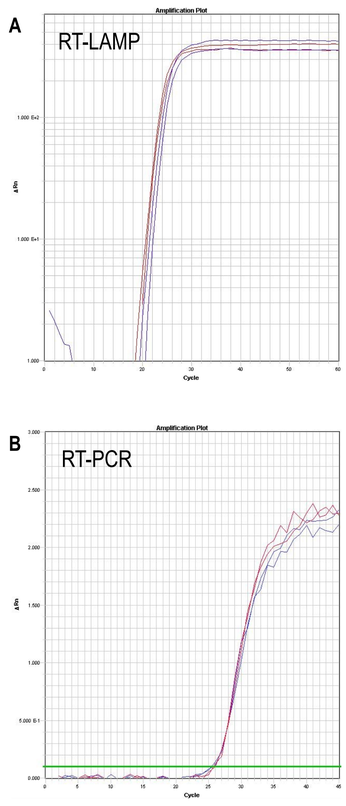

Fig. 2. Detection of *B. pseudomallei* in humus. Humic acid is present in the organic matter found in soil and is a known PCR amplification inhibitor. To determine if amplification is inhibited by humic acid on ARCIS-prepped samples, we first spiked humus, which has an organic matter content of close to 100%, with *B. pseudomallei*. RT-LAMP (A) and RT-PCR (B) were then performed with ARCIS-prepped DNA.

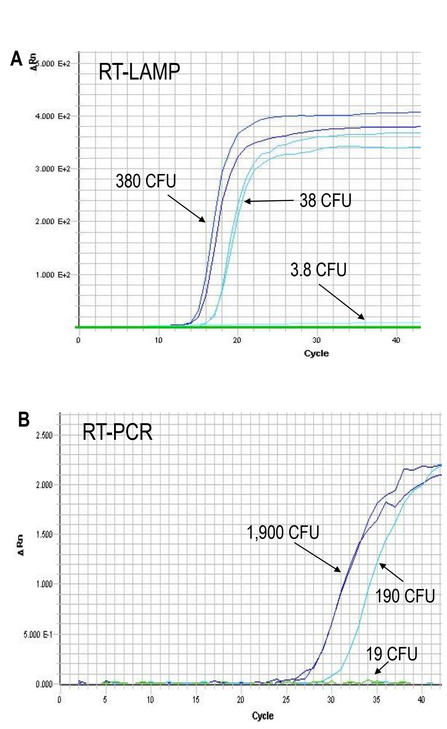

Fig. 3. *B. pseudomallei* limit of detection in SSL using ARCIS prep. A titration of *B. pseudomallei* was spiked into irradiated SSL soil samples, liquefied with 10 mM $CaCl_2$, and the DNA was prepared using the ARCIS kit. RT-LAMP and RT-PCR assays were performed. The LOD for RT-LAMP was estimated to be between 3.8 and 38 cells (A), and the LOD for RT-PCR was estimated to be between 19 and 190 cells (B).

Conclusions

- ARCIS Sample Prep Kit can be used to rapidly prepare DNA from soil for detection via RT-PCR and RT-LAMP and is comparable to the more labor intensive and less deployable DNeasy PowerSoil Kit.
- It appears the ARCIS Kit was able to neutralize the amplification inhibitors present in soil.
- ARCIS Kit is suitable for field-deployable detection and does not require cold chain or lab equipment such as a vortex or centrifuge.

Future Work

- Develop a highly sensitive and specific LAMP assay using *B. pseudomallei* consensus sequences.
- Incorporate filters and lyophilized ARCIS reagents into a microfluidic cassette (Fig. 4B).
- Integrate the microfluidic cassette into a field-deployable device (Fig 4A).
- Multiplex assay to include detection for other biothreat agents (Fig 4C).

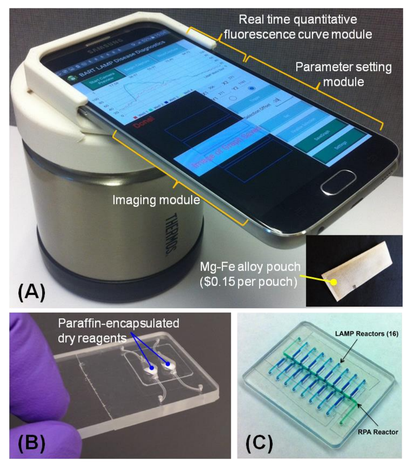

Fig. 4. Prototype of a highly multiplexed, miniature, field-deployable, sample-to-answer molecular detection system capable of co-detecting soil-borne pathogens.

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