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Mechanical Disruption of Lysis-Resistant Bacterial Cells by Use of a Miniature, Low-Power, Disposable Device▽†

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Molecular detection of microorganisms requires microbial cell disruption to release nucleic acids. Sensitive detection of thick-walled microorganisms such as *Bacillus* spores and *Mycobacterium* cells typically necessitates mechanical disruption through bead beating or sonication, using benchtop instruments that require line power. Miniaturized, low-power, battery-operated devices are needed to facilitate mechanical pathogen disruption for nucleic acid testing at the point of care and in field settings. We assessed the lysis efficiency of a very small disposable bead blender called OmniLyse relative to the industry standard benchtop Biospec Mini-BeadBeater. The OmniLyse weighs approximately 3 g, at a size of approximately 1.1 cm^3 without the battery pack. Both instruments were used to mechanically lyse *Bacillus subtilis* spores and *Mycobacterium bovis* BCG cells. The relative lysis efficiency was assessed through real-time PCR. Cycle threshold (C_T) values obtained at all microbial cell concentrations were similar between the two devices, indicating that the lysis efficiencies of the OmniLyse and the BioSpec Mini-BeadBeater were comparable. As an internal control, genomic DNA from a different organism was spiked at a constant concentration into each sample upstream of lysis. The C_T values for PCR amplification of lysed samples using primers specific to this internal control were comparable between the two devices, indicating negligible PCR inhibition or other secondary effects. Overall, the OmniLyse device was found to effectively lyse tough-walled organisms in a very small, disposable, battery-operated format, which is expected to facilitate sensitive point-of-care nucleic acid testing.

Nucleic acid testing has become an important tool in infectious disease diagnosis (4, 25), biothreat detection (14, 30), and research. Point-of-care or point-of-use applications of nucleic acid testing, especially in settings with minimal infrastructure, require novel tools that can perform essential tasks in miniaturized, inexpensive formats with the same performance characteristics as currently available, expensive, laboratory-based methods (13).

Lysis of an organism to liberate its genomic material is an important step in sample preparation for nucleic acid testing. Many common pathogens can be lysed through chemical agents, such as detergents and chaotropic salts, or by enzymatic treatment (8, 31). However, lysis is a significant challenge for thick-walled microorganisms such as *Bacillus anthracis* spores and *Mycobacterium tuberculosis* cells (13, 18, 22). The multilayer structure of *Bacillus* spores includes an outer cortex and coat that is resistant to chemical and physical treatments (5, 23). Similarly, mycobacteria have a thick, waxy cell wall that is difficult to disrupt for the extraction of nucleic acids (9, 17). High-energy mechanical disruption methods, such as sonication and bead beating, are commonly used for lysis of thick-walled organisms, since chemical, heat, freeze-thaw, or enzymatic lysis methods alone are less effective (1, 11, 22). Lysis protocols for mycobacteria have been reported that use low-energy bead beating (2, 6) in conjunction with heat or chemical or enzymatic lytic agents, which increase process complexity and potentially introduce PCR inhibitors. We are not aware of any published or unpublished methods that can break open slow-growing mycobacteria by low-energy bead beating alone, in the absence of other lytic treatments, with the same high efficiency as the BioSpec Mini-BeadBeater.

Disruption of thick-walled organisms by sonication typically involves the exposure of a suspension containing the pathogen and beads to high frequency sound waves that are delivered by a rapidly oscillating transducer. Lysis by sonication has been attributed to cavitation, where the rapid formation and shrinkage of gas bubbles creates high pressures and temperatures (5). Lysis of thick-walled organisms by bead beating typically involves high-frequency oscillation of a closed tube containing a suspension of the target organism and beads. The mechanism of lysis by bead-beating has been attributed to high shear rates between beads and strong periodic vortical flow fields (13). The diameter of beads used during mechanical lysis is critical to lysis efficiency, with 100-μm-diameter beads being more effective than larger-diameter beads at lysing Gram-positive bacteria (11, 22).

Bead beating and sonication typically require benchtop devices with significant power demands. The BioSpec Mini-BeadBeater (Fig. 1A) and the Sonics VibraCell Ultrasonic system are among the smallest devices on the market, at respective sizes of 3,900 cm^3 for the BioSpec Mini-BeadBeater and >7,400 cm^3 for the VibraCell Ultrasonic system, including the power supply (13). Larger, heavier, and more expensive bead-

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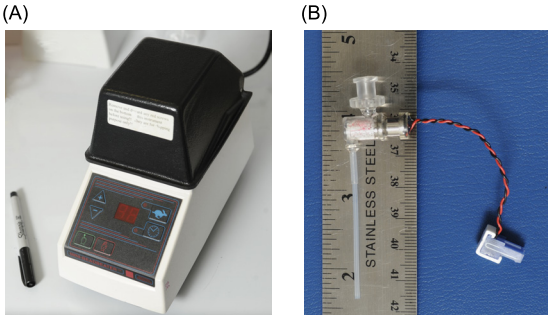

FIG. 1. Systems for mechanical disruption of tough-walled organisms. (A) Mini-BeadBeater (BioSpec), a typical benchtop instrument. (B) OmniLyse bead blender (Claremont BioSolutions), a miniaturized, disposable, battery-operated device.

beating devices exist, which can process multiple samples in parallel. The BioSpec Mini-BeadBeater device has been used in previous studies to lyse bacterial spores (13, 20). *Mycobacterium* cells are lysed effectively using the BioSpec Mini-BeadBeater (27) and Mini-BeadBeater-8 (12, 15). The BioSpec Mini-BeadBeater has been used as a standard to benchmark relative lysis efficiencies of new devices and techniques (13).

Fully integrated sample-to-answer nucleic acid testing systems are on the market, such as the Cepheid GeneXpert, which automates sample preparation through sonication, filtration, and solid-phase extraction, followed by amplification and detection via the real-time PCR (34). Mechanical cell disruption is facilitated through a miniaturized sonication device (5). GeneXpert cartridges are available to detect *B. anthracis* (34), *M. tuberculosis* (7, 16), and *Clostridium difficile* (26), three pathogens that are known to be lysis resistant. In the GeneXpert, sensitive detection of these tough-walled pathogens is facilitated through mechanical cell disruption via sonication.

Alternative methods for rapid mechanical lysis of thick-walled organisms have been developed at Keck Graduate Institute and are now commercialized by Claremont BioSolutions (13). One method uses a disposable, battery-operated/low-power, light weight, miniaturized, bead blender called the OmniLyse. This device (Fig. 1B) uses four AAA 1.5-V batteries, weighs approximately 3 g, and is 1.1 cm^3 in volume without a battery pack or 71 g and 44 cm^3 in volume with a battery pack. The OmniLyse consists of an injection-molded chamber with inlet and outlet ports that can be operated in batch or flowthrough mode and is therefore suitable for processing large sample volumes. A micro-motor equipped with a custom-designed precision-cut impellor is installed in such a way as to dissipate a high amount of mechanical energy within the chamber. The device utilizes a proprietary method for entrapping and retaining zirconia/silica beads within the lysis chamber during activation of the motor. High-speed camera video experiments have shown that the motor can drive the impellor at speeds greater than 30,000 rpm with the chamber filled with liquid and beads. The kinetic energy imparted onto the beads in solution generates high shear forces between beads, causing disruption of cells caught within the shear flow. The system is a single-use disposable unit, rendered feasible by use of inexpensive components.

We herein report a performance evaluation, assessing the lysis efficiency of the OmniLyse device relative to the industry standard benchtop Biospec Mini-BeadBeater (Fig. 1), using *B. subtilis* spores as a BSL1 surrogate for *B. anthracis* spores, and *M. bovis* BCG, an attenuated vaccine strain, as a BSL2 surrogate for *M. tuberculosis*.

MATERIALS AND METHODS

General reagents. Standard *Taq* buffer (B9014S), *Taq* DNA polymerase (M0273L), and deoxynucleotide triphosphate (dNTP; N0447S) were purchased from New England Biolabs. Magnesium chloride (ES25-100ml) was obtained from Amresco (Solon, OH). Uracil DNA glycosylase (catalog no. 78310) and PCR nucleotide mix with dUTP (catalog no. 77330) were acquired from USB (Cleveland, OH). Oligonucleotide primers were ordered from Integrated DNA Technologies (Coralville, IA) and Sigma-Aldrich (St. Louis, MO). SYBR green I (S7567) was acquired from Invitrogen (Carlsbad, CA). Power SYBR mix (catalog no. 4368702) was purchased from Applied Biosystems. Bovine serum albumin (BSA) was obtained from New England Biolabs (B9001S; Ipswich, MA) and Thermo Scientific (HyClone SH30574.03; Waltham, MA). TE buffer was composed of 10 mM Tris and 1 mM EDTA (pH 8.3). *B. subtilis* spores, strain GB03, were donated by Gustafson, LLC, now a subsidiary of Bayer Corp. *Aeromonas hydrophila* (strain 7966) was obtained from the American Type Culture Collection (Manassas, VA). The Kinyoun TB Stain Kit K (catalog no. 212315) was ordered from BD (Franklin Lakes, NJ). Gel electrophoresis to size double-stranded DNA following PCR was performed on the Agilent 2100 Bioanalyzer (Santa Clara, Ca) by using an Agilent DNA 1000 kit (catalog no. 5067-1504). The Mini-BeadBeater and the Mini-BeadBeater-8 were purchased from BioSpec (Bartlesville, OK).

OmniLyse device. Studies performed herein utilized the current version of Claremont BioSolutions (Upland, CA) OmniLyse device, as shown in Fig. 1B, with an inlet luer-lock fitting, a stiff outlet tube, and a lead-free micro-motor that in the current device configuration can operate at full speed for up to 20 min. The lysis chamber contains 200 mg of 100-μm-diameter acid-washed zirconia/silica beads. The OmniLyse device was operated using four AAA alkaline batteries at a nominal voltage of 6 V with a low electrical current between 100 and 200 mA. Since the typical capacity of an alkaline AAA battery is approximately 1,200 mAh, a single battery pack should operate between 6 and 12 h, which translates to an expected 250 to 500 lysis preparations of 1.5 min each per battery pack. This disposable lysis device is fabricated using inexpensive manufacturing methods. The main body and fittings of the device are produced through inexpensive injection molding. Also, the motors are mass produced in extremely high quantities and obtained at low cost. Even in low production volumes, Claremont BioSolutions is currently able to sell the devices within the price range of other disposables used in bioscience research. As volumes increase, both the cost of goods and the unit price are anticipated to drop precipitously.

Preparation of *B. subtilis spore* stocks. *B. subtilis* spores were suspended and washed three times in Nanopure filtered water. After the final wash and resuspension, the spores were quantified by using an iNcyto (Chungchongnam, Korea) C-chip hemocytometer (DHC-N01-2) and through colony counting using LB agar plates. A Nikon Eclipse TE2000-S microscope was used to view microbial samples. Both methods resulted in similar spore counts. The Schaeffer-Fulton staining method (32) was used to confirm that the *B. subtilis* microorganisms were present as spores and not as vegetative cells. The spore stocks were stored at −20°C until further use.

Lysis of *B. subtilis* spores. Dilution series of *B. subtilis* spores ranging from 10^4 to 10^8 spores/ml in the first experiment and from 10^3 to 10^8 spores/ml in the second experiment were prepared in TE buffer, to which 10^6 copies of purified *Escherichia coli* K-12 genomic DNA/ml was added as an internal control. (*E. coli* K-12 was grown in nutrient broth at 37°C, and the DNA was purified and extracted by using a Qiagen [Venlo, Netherlands] DNeasy Blood & Tissue Kit [catalog no. 69504].) Each sample in this dilution series was processed in triplicate by using the OmniLyse device and the BioSpec Mini-BeadBeater. The same 100-μm-diameter zirconia/silica beads were used in both devices. For lysis using the OmniLyse device, 500-μl sample aliquots were aspirated from the sample tube through the stiff tubing into the device and into a syringe attached at the port with the luer lock and then dispensed back through the device into the sample tube for a total of three passes, with a total processing time per sample of 1.5 min. Each replicate was performed with a new device. Crude lysates were kept on ice until samples were tested using PCR. For lysis using the BioSpec Mini-BeadBeater, 625-μl sample aliquots plus 250 mg of 100-μm-diameter zirconia/silica beads were added to 2-ml conical tubes. The samples were bead beaten at

TABLE 1. Sequences of forward and reverse primers used during PCR

Target organism	Primer sequence (5′–3′)		Product size (bp)	Reference
	Forward	Reverse		
B. subtilis	GTTTTGTTCTTTTCCTGTGCC	GCTTCCAGCTTACTGATATCC	128	28
E. coli	ACGCTGCCCGATATAACAAC	GCAATGGCGTAAAAATTGGT	236	21
A. hydrophila	ATTGAGCCGCCTTAACAGG	AACTGTTATCCCCCTCGAC	189	10
M. bovis BCG	CCTGCGAGCGTAGGCGTC	CTCGTCCAGCGCCGCTTC	123	3[a]

[a] The oligonucleotide primer sequence was modified from reference 3.

maximum speed (4,800 rpm) for 1-min pulses, three times, with a 30-s rest on ice between pulses. When finished, the samples were immediately transferred to fresh tubes and kept on ice until testing by PCR.

PCR analysis of *B. subtilis* samples. After processing, each sample was split and interrogated with two primer sets, one specific for *B. subtilis* and the other specific for *E. coli* DNA (Table 1). Then, 12.5 μl of sample was added to 37.5 μl of master mix. The thermocycling conditions consisted of a 10-min 94°C hot start, followed by 45 cycles of 94°C for 8 s, 58.7°C for 15 s, and 72°C for 28 s. For the second experiment, a 37°C 10-min UNG (uracil *N*-glycosylase) step was added before the 10-min 94°C hot start step. Each PCR mixture contained 1× standard *Taq* buffer, 0.25 mM concentrations of dNTPs, 0.08 U of *Taq* Polymerase/μl, 4.5 mM $MgCl_2$, 0.3 μM concentrations of the forward and reverse (F/R) primers, 0.8 mg of BSA/ml, and 1× SYBR green I. For the second test dUTPs replaced the dTTPs (at the same final concentration) and 0.008 U of UNG/μl was added to the master mix. PCR was performed using the DNA Engine Opticon 2 (Bio-Rad, Hercules, CA). For the lysis control experiments, each PCR sample contained 0.3 μM concentrations of the F/R primers, 0.8 mg of BSA/ml, and 1× Power SYBR green PCR mix.

Cultivation of *M. bovis* BCG. *Mycobacterium bovis* BCG strain Russia was grown in Difco Middlebrook 7H9 broth medium supplemented with 10% albumin-dextrose-catalase enrichment (BBL), 0.2% glycerol, and 0.05% Tween 80 at 37°C. Cultures were maintained in log-phase growth (optical density at 600 nm [OD_{600}] = 0.2 to 1.0, where 1 OD_{600} = 4.5E8 cells/ml) before use in experiments. The cells were pelleted by centrifugation at 16,000 × *g* for 1 min or 3,000 × *g* for 30 min and washed once with 1× phosphate-buffered saline (equivalent culture volume) and resuspended in TE to the desired concentration. A Kinyoun TB Stain Kit K was used to confirm the presence of *Mycobacterium* cells.

Lysis of *M. bovis* BCG cells. A dilution series of *M. bovis* BCG, in TE buffer containing 10^6 copies of purified *Aeromonas hydrophila* genomic DNA/ml, was prepared ranging from 10^4 to 10^8 CFU/ml in the first experiment and from 10^3 to 10^8 CFU/ml in the second experiment. (*A. hydrophila* was grown on nutrient agar or in nutrient broth at 28 to 30°C, and the DNA was purified and extracted by using a MoBio Laboratories [Carlsbad, CA] UltraClean microbial DNA isolation kit [10].) Then, 4 mg of BSA/ml was added upstream to the TE buffer in the second experiment. Triplicates of samples in the dilution series were processed using either the OmniLyse device or the BioSpec Mini-BeadBeater-8. For lysis using the OmniLyse device, portions (1 ml in the first experiment and 500 μl in the second experiment) of the samples were aspirated from the sample tube through the stiff tubing into the device and into a syringe attached at the port with the luer lock and then dispensed back through the device into the sample tube for a total of three passes, with a total processing time per sample of 1.5 min. The experiment was performed in triplicates, using a new device for each replicate. For lysis using the BioSpec Mini-BeadBeater-8 device, 1.2 ml or 625 μl in the first and second experiments, respectively, plus 250 mg of beads, was added to 2-ml conical tubes. For the BioSpec Mini-BeadBeater-8, Sigma-Aldrich 106-μm-diameter glass beads (G-4649) were used in the first experiment, and the same 100-μm-diameter zirconia/silica beads used in the OmniLyse blender were used in the second experiment. The samples were bead beaten at maximum speed (2,800 rpm) for 1-min pulses, three times, with a 30-s rest on ice between pulses (10, 33). Crude lysates were stored at −80°C after processing until tested using PCR. A control experiment was also performed in which processed samples were split and either frozen at −80°C or kept on ice until tested using PCR.

PCR analysis of *M. bovis* BCG samples. After processing, each sample was split and interrogated with two primer sets, one specific for *M. bovis* BCG and the other specific for *A. hydrophila* DNA (Table 1). For PCR analyses, 5 μl of sample was added to 20 μl of master mix. Each PCR sample contained the F/R primers 0.3 μM and 1× Power SYBR green PCR mix. In experiment 1, each PCR sample further contained 0.8 mg of BSA/ml in the master mix. The thermocycling conditions consisted of a 10-min 95°C hot start, followed by 45 cycles of 95°C for 15 s, 59°C for 30 s, and 72°C for 30 s. A dissociation curve between 95 and 65°C was acquired to confirm the identity of the amplicons. PCR was performed using an Applied Biosystems 7500 Real-Time instrument (Foster City, CA).

RESULTS

We evaluated the capability of the OmniLyse device to mechanically disrupt thick-walled organisms and liberate DNA suitable for PCR amplification, relative to the BioSpec Mini-BeadBeater and Mini BeadBeater-8, two industry-standard benchtop mechanical lysis devices. Figure 2 provides an overview of the experiments performed. To the extent possible, we used similar conditions in the OmniLyse and the BioSpec devices. Unless otherwise stated, all data points represent the averaged cycle threshold (C_T) values of triplicate runs, with the error bars representing the standard deviation. In all experiments, we included triplicates of a control sample that only contained a fixed concentration of the internal control DNA but none of the targeted microorganism DNA. Since the *x* axes in Fig. 3 to 5 represent the concentrations of the targeted microorganisms, this control sample was added as an additional point at 0 CFU/ml.

Lysis efficiency. The targeted microorganisms were *B. subtilis* spores or *M. bovis* BCG cells. The C_T values obtained from PCRs with primers specific for each organism were used as indicators of lysis efficiency. If both devices have similar lysis efficiencies, then similar C_T values should be obtained at each concentration of the targeted microorganism. In general, *B.*

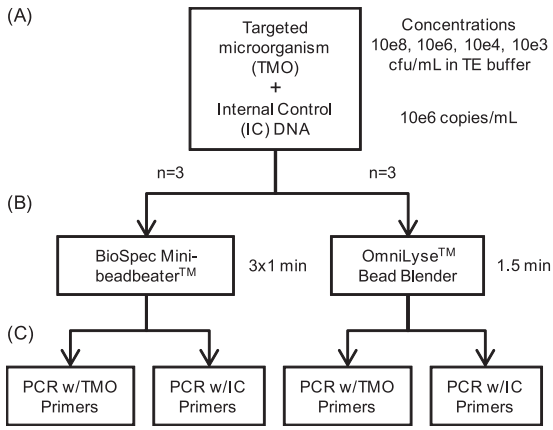

FIG. 2. Procedure overview. (A and B) Serial dilutions of the targeted microorganism (TMO) plus a fixed amount of internal control (IC) genomic DNA were processed in triplicate using an OmniLyse device for 1.5 min or a BioSpec Mini-BeadBeater/Mini-BeadBeater-8 in three 1-min pulses, with 30-s rests on ice between pulses. (C) Processed samples were split and analyzed by PCR using primers either specific for the TMO or the IC.

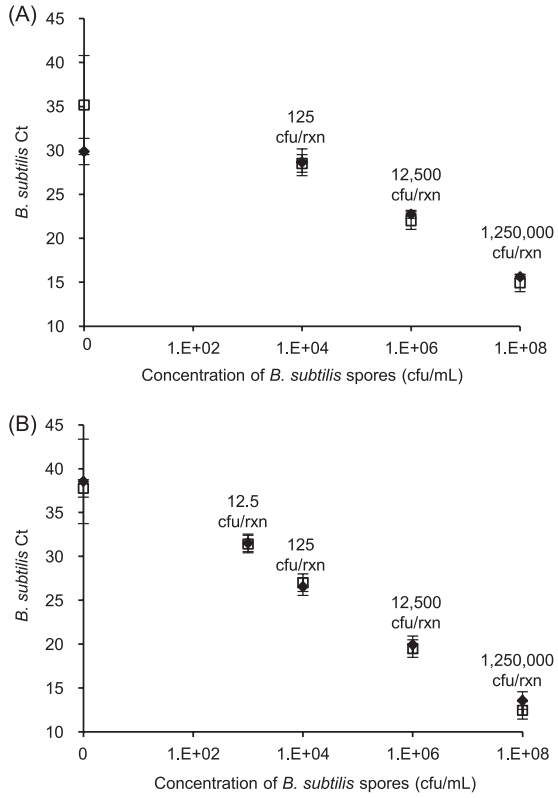

FIG. 3. Lysis of *B. subtilis* spores. PCR C_T values are shown for solutions containing spores at different concentrations, processed using either the OmniLyse device (◆) or the BioSpec Mini-BeadBeater (□), with primers specific to *B. subtilis*. The numbers for CFU per reaction (CFU/rxn) indicated in the graph are theoretical maxima, based on 12.5 μl of lysed sample added to each PCR, and assume no loss during sample processing. In both experiments A and B, the C_T values of spores lysed obtained using either device were comparable, indicating similar lysis efficiency. During experiment A, the no-target control (0 CFU/ml) samples appeared earlier than expected. Gel electrophoresis results (data not shown) suggested that this was predominantly due to primer dimers.

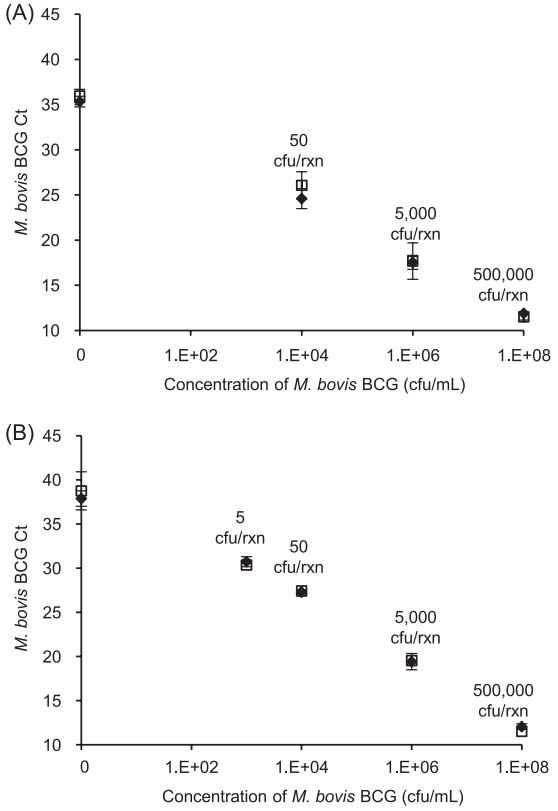

FIG. 4. Lysis of *M. bovis* BCG cells. C_T values are shown for solutions containing *M. bovis* BCG at different concentrations, processed using either the OmniLyse device (◆) or the BioSpec Mini-BeadBeater (□), with primers specific to *M. bovis* BCG. The numbers for CFU/rxn indicated in the graph are theoretical maxima, based on 5 μl of lysed sample added to each PCR, and assume no loss during sample processing. Since mycobacteria are prone to clumping, one CFU may represent more than one cell or genome. In both experiments A and B, the C_T values of BCG cells lysed using either device were comparable, indicating similar lysis efficiency. For experiment A, only one replicate was tested for the 10^8 CFU/ml concentration using the OmniLyse blender. In experiment A, glass beads were used in the BioSpec device, while zirconia/silica beads were used in all other experiments.

subtilis spores processed through either the OmniLyse device or the BioSpec Mini-BeadBeater, quantified by using *B. subtilis*-specific primers, yielded comparable C_T values at all concentrations tested in two independent experiments (Fig. 3). Likewise, *M. bovis* BCG cells processed through either the OmniLyse device or the BioSpec Mini-BeadBeater, using *M. bovis* BCG-specific primers, resulted in comparable C_T values at all concentrations tested in two independent experiments (Fig. 4). Since the purpose of the present study is to determine a difference in C_T value as function of device, independent of the targeted microorganism's concentration, the sample size per device includes all replicates for all concentrations and both experiments. This experimental design has a statistical power of >90% for predicting a significant ΔC_T of 0.5 between the two devices, with a standard deviation in C_T of 0.5 (signal-to-noise ratio [S/N] = 1). Based on a two-factor analysis of variance (ANOVA, see Fig. S1 in the supplemental material), there is no significant difference between the C_T values (as indicators of lysis efficiency) obtained using the BioSpec versus those obtained using the OmniLyse device, for either *B. subtilis* spore lysis ($P = 0.3225$) or lysis of *M. bovis* BCG ($P = 0.7845$). This ANOVA treated the targeted microorganism concentration as factor 1 and lysis device as factor 2, using a blocked experimental design, wherein experimental sets (Fig. 3 and 4) are treated as blocks.

In the first experiment (Fig. 4A), glass beads were used in the BioSpec device and zirconia/silica beads were used in the OmniLyse blender. In the second experiment (Fig. 4B), zirconia/silica beads were used in both devices. The C_T values obtained using the BioSpec device are almost identical in both experiments, indicating that the bead type used does not appreciably affect the lysis efficiency.

In lysis experiments of *B. subtilis* spores and *M. bovis* BCG cells, averaged C_T values over the range of concentrations tested for both devices were within a standard deviation of each other. Having BSA present in the master mix was found to be important for obtaining robust and reproducible experimental results when zirconia/silica beads were used in either device, especially at lower BCG cell concentrations, as dis-

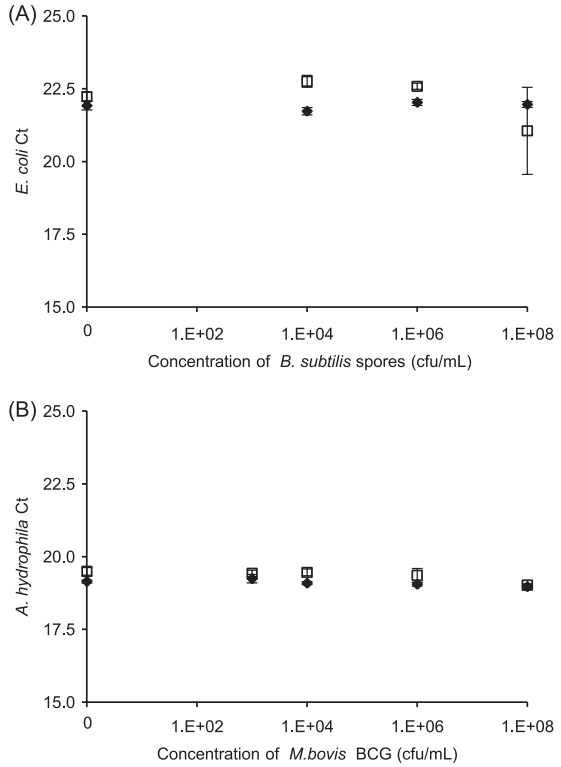

FIG. 5. Assessment of secondary effects on PCR amplification of internal control DNA. (A) C_T values for amplification of *E. coli* genomic DNA spiked as an internal control at a constant concentration in *B. subtilis* suspensions of various concentrations, processed via the OmniLyse (◆) or BioSpec (□) device. Based on a 12.5-μl sample added per PCR and assuming no loss during processing, each PCR should contain 12,500 copies *E. coli* genomic DNA. (B) C_T values for amplification of *A. hydrophila* genomic DNA spiked as an internal control at a constant concentration into *M. bovis* BCG suspensions of various concentrations, processed via the OmniLyse (◆) or BioSpec (□) device. Based on a 5-μl sample added per PCR, and assuming no loss during processing, each PCR should contain 5,000 copies *A. hydrophila* genomic DNA. Overall, the C_T values of the internal controls are reasonably comparable for the OmniLyse device and the BioSpec Mini-BeadBeater devices, and are independent of the concentration of the targeted microorganism.

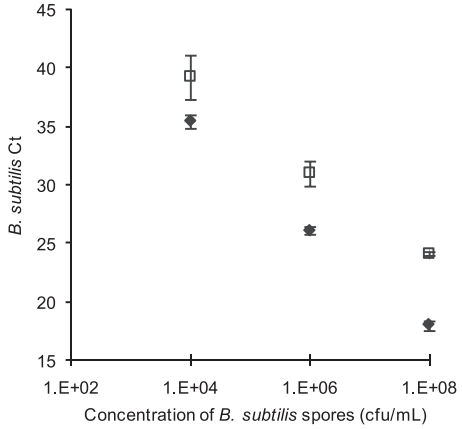

FIG. 6. Control experiment to differentiate detection of *B. subtilis* extracellular DNA from detection of DNA as result of *B. subtilis* spore lysis. Samples lysed using the BioSpec system followed by PCR analysis (◆) amplify approximately 4 to 6 C_T values earlier than samples containing the same concentration of *B. subtilis* spores but analyzed via PCR without the mechanical disruption step (□). Mechanical lysis results in 10- to 100-fold more PCR-amplifiable target DNA, assuming a PCR efficiency of ca. 100%.

cussed in below. When glass beads were used in the Biospec device, robust and reproducible results were obtained regardless of the cell concentration or presence of BSA.

B. subtilis spores are known to carry extracellular DNA (5, 19). To rule out the possibility that PCR experiments predominantly detected extracellular DNA, we conducted a control experiment. *B. subtilis* spores at concentrations of 10^4, 10^6, and 10^8 CFU/ml were either lysed using the BioSpec system, followed by PCR analysis, or analyzed via PCR without the mechanical disruption step. At these concentrations, the unprocessed spores amplified approximately 4 to 6 C_T values later than the samples that underwent mechanical lysis (Fig. 6). A similar experiment was performed for *M. bovis* BCG cells at concentrations of 10^8 and 10^6 CFU/ml (see Fig. S2A in the supplemental material). Again, the unprocessed bacterial cells amplified approximately 4 to 6 C_T values later than the samples that underwent mechanical lysis. Therefore, mechanical lysis results in 10- to 100-fold more PCR-amplifiable target DNA, assuming a PCR efficiency of ca. 100%. The true difference is likely greater since unprocessed control samples probably underwent partial heat lysis during the 10-min, 95°C PCR hot start step. In another experiment, intact *M. bovis* BCG cells were removed from processed and unprocessed samples by centrifugation prior to PCR. In this case, unprocessed samples amplified ~10 C_T values later than processed samples (see Fig. S2B in the supplemental material).

For the *M. bovis* BCG study, lysed samples were frozen at −80°C prior to PCR processing. To investigate whether this freeze-thaw step artificially enhanced mycobacterial lysis, we conducted a separate experiment, wherein *M. bovis* BCG samples lysed using the BioSpec device were either kept on ice or frozen at −80°C prior to PCR processing. The C_T values obtained using both processing methods were comparable (see Fig. S2A in the supplemental material), and averages were within a standard deviation of each other.

Internal control results. To assess secondary effects such as DNA degradation and PCR inhibition, we added an internal control (IC) to each reaction upstream of the lysis step, consisting of a fixed amount of purified genomic DNA from a different organism. If there are no secondary effects or if unaccounted secondary effects are similar for both devices and at each concentration of the targeted microorganism, then the IC C_T values should be comparable and relatively constant.

For the *B. subtilis* lysis experiment, *E. coli* genomic DNA was added as an IC and processed by PCR for the first experiment only, as shown in Fig. 5A. For the *M. bovis* BCG lysis experiments, *A. hydrophila* genomic DNA was added as an IC and processed by PCR in both experiments. The results for IC amplification were similar in both experiments; data for the second experiment are shown in Fig. 5B.

Overall, the C_T values of the internal controls were comparable for the OmniLyse device and the BioSpec Mini-BeadBeaters. Cross-amplification, i.e., amplification of the DNA from the targeted microorganism using IC-specific primers and

vice versa, was not observed in either case. In the *B. subtilis* and *M. bovis* BCG experiments, correct amplicon formation was confirmed via gel electrophoresis and melting-curve analysis, respectively. Amplification with *B. subtilis* specific primers for some of the lower *B. subtilis* concentrations and the no-target controls also contained primer dimers.

The presence of BSA in the master mix appeared to be critical to obtaining reproducible and robust C_T values following mechanical lysis using the zirconia/silica beads in either the OmniLyse or the BioSpec Mini-BeadBeater-8. When BSA was omitted in initial experiments with *M. bovis* BCG, the C_T values were inconsistent, with many failed amplifications, especially at the lower *M. bovis* BCG concentrations. The C_T values of the IC (*A. hydrophila* genomic DNA) increased with decreasing *M. bovis* BCG concentration. Late amplification was also observed in the no-target control, containing *A. hydrophila* DNA only in buffer that was processed through the respective bead beating device. Dilution series of the purified *A. hydrophila* DNA not processed through a lysis device did not show this effect. Adding BSA directly to the bacterial suspension prior to lysis, or to the master mix following lysis, eliminated these effects and enabled consistent results.

DISCUSSION

The miniaturized OmniLyse device mechanically lyses *B. subtilis* spores and *M. bovis* BCG cells with efficacy similar to the industry-standard benchtop BioSpec Mini-BeadBeater, based on the comparable C_T values obtained from PCRs with primers specific for these two microorganisms. *Bacillus* spores carry extracellular DNA that may be bound on the bacterial spore coats. This extracellular DNA is not readily removed by multiple wash steps and can be amplified during PCR even without mechanical lysis (5, 19). Although it is possible that extracellular DNA was coamplified in the experiments described here, a control experiment demonstrated that mechanical lysis significantly increased (between 10- and 100-fold) the amount of PCR-amplifiable target DNA, a finding in line with the expectation that mechanical lysis is required to achieve a low limit of detection. In addition, a previous time series lysis study of DNase-treated *B. subtilis* spores using the OmniLyse device clearly demonstrated that this device mechanically disrupts spores and liberates intracellular DNA (13). Likewise, other studies have confirmed the ability of the BioSpec Mini-BeadBeater to lyse *Bacillus* spores (20, 24).

Using the protocol described here, the experiments did not appear to be confounded by secondary effects such as DNA degradation or PCR inhibition, based on consistent internal control C_T values obtained for both devices and at each concentration of the targeted microorganism. For lysis experiments using zirconia/silica beads in either device, the presence of BSA in the master mix was required for consistent, reproducible results. Although further experiments are required to fully understand this effect, it is possible that BSA relieves a PCR-inhibitory effect linked to fragments of zirconia/silica beads carried into the PCR. This hypothesis is supported by the observation that when glass beads were used in the BioSpec device in place of zirconia/silica beads, no inhibition was observed, and reproducible results were obtained at all cell concentrations with or without BSA. BSA is commonly used as a PCR facilitator (29, 36), and it may bind to fragments of zirconia/silica beads carried into the PCR. The inhibitory effect was unlikely to be caused by cellular debris because the effect was less prominent at higher concentrations of the targeted microorganism and was also observed in the no-target controls containing IC DNA only in buffer processed through a bead-beating device.

To the extent possible, we used similar conditions in the OmniLyse and the BioSpec devices, which included the use of similar or identical beads and bead-to-volume ratios. The processing time per sample for the OmniLyse device (1.5 min without rest), however, was significantly less than for the BioSpec BeadBeater devices (three 1-min pulses with 30-s rests between pulses). The BioSpec processing time used in the present study was based on standard published protocols (10, 13, 33, 35), and appears to be the time required using this system for effective lysis of *Bacillus* spores and mycobacterial cells. In most experiments, the same zirconia/silica beads were used in both devices. BioSpec protocols reported in the literature use either glass beads or zirconia/silica beads for lysis of mycobacteria (22, 33, 35). Based on our results (Fig. 4), the use of glass beads versus zirconia/silica beads in the BioSpec did not appreciably affect the lysis efficiency using this device and did not alter the relative lysis efficiency compared to the OmniLyse.

In summary, for both lysis-resistant bacterial strains studied, mechanical lysis using the OmniLyse blender and the BioSpec Mini-BeadBeaters resulted in similar C_T values for PCR analysis of processed samples over the range of concentrations studied. Amplification of IC DNA spiked at a constant concentration into the samples upstream of lysis using either device resulted in consistent PCR C_T values for all processed samples. Overall, the OmniLyse device facilitates effective lysis of tough-walled organisms in a miniaturized, disposable, battery-operated format, with an efficiency comparable to that of a widely used benchtop system. Future work will focus on combining lysis with extraction of nucleic acids within the bead blender with suitable nucleic acid amplification and detection methods in a fully integrated cartridge for handheld point-of-care nucleic acid testing in low-resource settings.

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P.E.V., K.M.W., B.E., G.A.C., and A.N. declare that they have no conflicts of interest. R.D., A.N., and B.I. each own a minority interest in Claremont BioSolutions, LLC. J.S. works on a part-time basis for Claremont BioSolutions, LLC.

REFERENCES

1. **Amaro, A., E. Duarte, A. Amado, H. Ferronha, and A. Botelho.** 2008. Comparison of three DNA extraction methods for *Mycobacterium bovis*, *Mycobacterium tuberculosis*, and *Mycobacterium avium* subsp. *avium.* Lett. Appl. Microbiol. **47:**8–11.
2. **Anderberg, R. J., J. A. Strachan, and G. A. Cangelosi.** 1995. Purification of DNA from *Mycobacterium* species without sonication or phenol. Biotechniques **18:**217–219.
3. **Balamurugan, R., S. Venkataraman, K. R. John, and B. S. Ramakrishna.** 2006. PCR amplification of the IS*6110* insertion element of *Mycobacterium*

tuberculosis in fecal samples from patients with intestinal tuberculosis. J. Clin. Microbiol. **44:**1884–1886.
4. **Barken, K. B., J. A. J. Haagensen, and T. Tolker-Nielsen.** 2007. Advances in nucleic acid-based diagnostics of bacterial infections. Clin. Chim. Acta **384:** 1–11.
5. **Belgrader, P., et al.** 1999. A minisonicator to rapidly disrupt bacterial spores for DNA analysis. Anal. Chem. **71:**4232–4236.
6. **Belisle, J. T., S. B. Mahaffey, and P. J. Hill.** 2009. Isolation of *Mycobacterium* species genomic DNA, p. 1–12. *In* T. Parish and A. C. Brown (ed.), Mycobacteria protocols, 2nd ed., vol. 465. Humana Press, Inc., Totowa, NJ.
7. **Boehme, C. C., et al.** 2010. Rapid molecular detection of tuberculosis and rifampin resistance. N. Engl. J. Med. **363:**1005–1015.
8. **Boom, R., et al.** 1990. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. **28:**495–503.
9. **Brennan, P. J., and H. Nikaido.** 1995. The envelope of mycobacteria. Annu. Rev. Biochem. **64:**29–63.
10. **Cangelosi, G. A., K. M. Weigel, C. Lefthand-Begay, and J. S. Meschke.** 2010. Molecular detection of viable bacterial pathogens in water by ratiometric pre-rRNA analysis. Appl. Environ. Microbiol. **76:**960–962.
11. **de Boer, R., et al.** 2010. Improved detection of microbial DNA after bead-beating before DNA isolation. J. Microbiol. Methods **80:**209–211.
12. **Dhandayuthapani, S., Y. Zhang, M. H. Mudd, and V. Deretic.** 1996. Oxidative stress response and its role in sensitivity to isoniazid in mycobacteria: characterization and inducibility of *ahpC* by peroxides in *Mycobacterium smegmatis* and lack of expression in *M. aurum* and *M. tuberculosis*. J. Bacteriol. **178:**3641–3649.
13. **Doebler, R. W., et al.** 2009. Continuous-flow, rapid lysis devices for biodefense nucleic acid diagnostic systems. J. Assoc. Lab. Automation **14:**119–125.
14. **Fan, J., A. J. Kraft, and K. J. Henrickson.** 2006. Current methods for the rapid diagnosis of bioterrorism-related infectious agents. Pediatr. Clin. N. Am. **53:**817.
15. **George, K. M., Y. Yuan, D. R. Sherman, and C. E. Barry.** 1995. The biosynthesis of cyclopropanated mycolic acids in *Mycobacterium tuberculosis*: identification and functional analysis of CMAS-2. J. Biol. Chem. **270:**27292–27298.
16. **Helb, D., et al.** 2010. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. J. Clin. Microbiol. **48:**229–237.
17. **Hoffmann, C., A. Leis, M. Niederweis, J. M. Plitzko, and H. Engelhardt.** 2008. Disclosure of the mycobacterial outer membrane: cryo-electron tomography and vitreous sections reveal the lipid bilayer structure. Proc. Natl. Acad. Sci. U. S. A. **105:**3963–3967.
18. **Hurley, S. S., G. A. Splitter, and R. A. Welch.** 1987. Rapid lysis technique for mycobacterial species. J. Clin. Microbiol. **25:**2227–2229.
19. **Johns, M., L. Harrington, R. W. Titball, and D. L. Leslie.** 1994. Improved methods for the detection of *Bacillus anthracis* spores by the polymerase chain reaction. Lett. Appl. Microbiol. **18:**236–238.
20. **Jones, C. A., N. L. Padula, and P. Setlow.** 2005. Effect of mechanical abrasion on the viability, disruption and germination of spores of *Bacillus subtilis*. J. Appl. Microbiol. **99:**1484–1494.
21. **Kamei, T., et al.** 2005. Microfluidic genetic analysis with an integrated a-Si:H detector. Biomed. Microdevices **7:**147–152.
22. **Kaser, M., M. T. Ruf, J. Hauser, L. Marsollier, and G. Pluschke.** 2009. Optimized method for preparation of DNA from pathogenic and environmental mycobacteria. Appl. Environ. Microbiol. **75:**414–418.
23. **Kazakov, S., E. Bonvouloir, and I. Gazaryan.** 2008. Physicochemical characterization of natural ionic microreservoirs: *Bacillus subtilis* dormant spores. J. Phys. Chem. B **112:**2233–2244.
24. **Longchamp, P., and T. Leighton.** 1999. Molecular recognition specificity of *Bacillus anthracis* spore antibodies. J. Appl. Microbiol. **87:**246–249.
25. **Millar, B. C., J. R. Xu, and J. E. Moore.** 2007. Molecular diagnostics of medically important bacterial infections. Curr. Issues Mol. Biol. **9:**21–39.
26. **Novak-Weekley, S. M., et al.** 2010. *Clostridium difficile* testing in the clinical laboratory by use of multiple testing algorithms. J. Clin. Microbiol. **48:**889–893.
27. **Odumeru, J., A. Gao, S. Chen, M. Raymond, and L. Mutharia.** 2001. Use of the bead beater for preparation of *Mycobacterium paratuberculosis* template DNA in milk. Can. J. Vet. Res.-Rev. Can. Recherche Vet. **65:**201–205.
28. **Pemov, A., H. Modi, D. P. Chandler, and S. Bavykin.** 2005. DNA analysis with multiplex microarray-enhanced PCR. Nucleic Acids Res. **33:**e11.
29. **Radstrom, P., R. Knutsson, P. Wolffs, M. Lovenklev, and C. Lofstrom.** 2004. Pre-PCR processing: strategies to generate PCR-compatible samples. Mol. Biotechnol. **26:**133–146.
30. **Rao, S. S., K. V. Mohan, and C. D. Atreya.** 2010. Detection technologies for *Bacillus anthracis*: prospects and challenges. J. Microbiol. Methods **82:**1–10.
31. **Salazar, O., and J. A. Asenjo.** 2007. Enzymatic lysis of microbial cells. Biotechnol. Lett. **29:**985–994.
32. **Schaeffer, A. B., and M. D. Fulton.** 1933. A simplified method of staining endospores. Science **77:**194.
33. **Sherman, D. R., et al.** 2001. Regulation of the *Mycobacterium tuberculosis* hypoxic response gene encoding alpha-crystallin. Proc. Natl. Acad. Sci. U. S. A. **98:**7534–7539.
34. **Ulrich, M. P., et al.** 2006. Evaluation of the Cepheid GeneXpert (R) system for detecting *Bacillus anthracis*. J. Appl. Microbiol. **100:**1011–1016.
35. **Via, L. E., and J. O. Falkinham.** 1995. Comparison of methods for isolation of *Mycobacterium avium* complex DNA for use in PCR and rapid fingerprinting. J. Microbiol. Methods **21:**151–161.
36. **Wilson, I. G.** 1997. Inhibition and facilitation of nucleic acid amplification. Appl. Environ. Microbiol. **63:**3741–3751.