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Comparison of point-of-care-compatible lysis methods for bacteria and viruses

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ABSTRACT

Nucleic acid sample preparation has been an especially challenging barrier to point-of-care nucleic acid amplification tests in low-resource settings. Here we provide a head-to-head comparison of methods for lysis of, and nucleic acid release from, several pathogenic bacteria and viruses—methods that are adaptable to point-of-care usage in low-resource settings. Digestion with achromopeptidase, a mixture of proteases and peptidoglycan-specific hydrolases, followed by thermal deactivation in a boiling water bath, effectively released amplifiable nucleic acid from *Staphylococcus aureus, Bordetella pertussis,* respiratory syncytial virus, and influenza virus. Achromopeptidase was functional after dehydration and reconstitution, even after eleven months of dry storage without refrigeration. Mechanical lysis methods proved to be effective against a hard-to-lyse *Mycobacterium* species, and a miniature bead-mill, the AudioLyse, is shown to be capable of releasing amplifiable DNA and RNA from this species. We conclude that point-of-care-compatible sample preparation methods for nucleic acid tests need not introduce amplification inhibitors, and can provide amplification-ready lysates from a wide range of bacterial and viral pathogens.

1. Introduction

In the modern clinical laboratory, detection of a myriad of pathogens with extreme sensitivity and specificity allows rapid diagnosis of disease and recommendations for treatment. However, these techniques are not always compatible with low-resource settings, or the point-of-care (POC). This disparity in technology availability affects not only health outcomes, but increases the burden of infectious disease in laboratory-poor areas of the developing world (Yager et al., 2006). In order to narrow this divide, our group is developing the MAD NAAT (Multiplexable Autonomous Disposable for Nucleic Acid Amplification Testing) platform (Fig. 1A). The launch point for the MAD NAAT project was the use of two-dimensional networks of paper (2DPNs) to automate an isothermal nucleic acid (NA) amplification process, isothermal strand displacement amplification (iSDA) (Toley et al., 2015b). To enable full sample-to result NAAT automation, advances were required with regard to sample acquisition and transfer into the device (Panpradist et al., 2014), valving to automate and sequence the assay (Toley et al., 2015a; Toley et al., 2013), nucleic acid purification and concentration (Byrnes et al., 2015), isothermal nucleic acid amplification chemistry (Toley et al., 2015b), and non-electric temperature-regulated heaters (Buser et al., 2015a; Shah et al., 2015; Singleton et al., 2013). Most sample preparation techniques in the literature are predicated upon use in a laboratory. Point-of-care-compatible methods have previously been described in isolation; to evaluate nucleic acid preparation efficacy they must be compared head-to-head.

A sample preparation protocol must consider a number of constraints to be compatible with a lab-on-a-chip (LOC) device such as the MAD NAAT, informed by conditions found in the developing world where many of the mainstays of laboratory methods are absent (Archibald and Reller, 2001; Mabey et al., 2004). In these environments, trained personnel are rare. Electricity, and thus equipment such as refrigerators and centrifuges, may be unavailable or unreliable, and disposables such as pipette tips and centrifuge tubes may be cost-prohibitive. The same constraints are found in environmental monitoring and diagnosis of veterinary and plant diseases in the field. Currently available rapid diagnostics for non-human pathogens detect pathogen-specific antibodies or antigens using immunochemistry (Yetisen et al., 2013). Nucleic acid amplification tests are capable of low-copy detection, leading to greater sensitivity for the target of interest, but are often also more sensitive to inhibitors present in the specimen (Rådström et al., 2004). Currently, NAAT technologies for non-human pathogens are under development, but none is commercially available in a sample-to-result format (Biswas and Sakai, 2014; Teles and Fonseca, 2015).

Constraints on diagnostics at the point-of-care are encompassed by the World Health Organization's ASSURED criteria: tests must be **A**ffordable, **S**ensitive, **S**pecific, **U**ser-friendly, **R**apid and robust, **E**quipment-free, and **D**elivered to those in need (Mabey et al., 2004). In order to meet these conditions for the MAD NAAT platform, we sought

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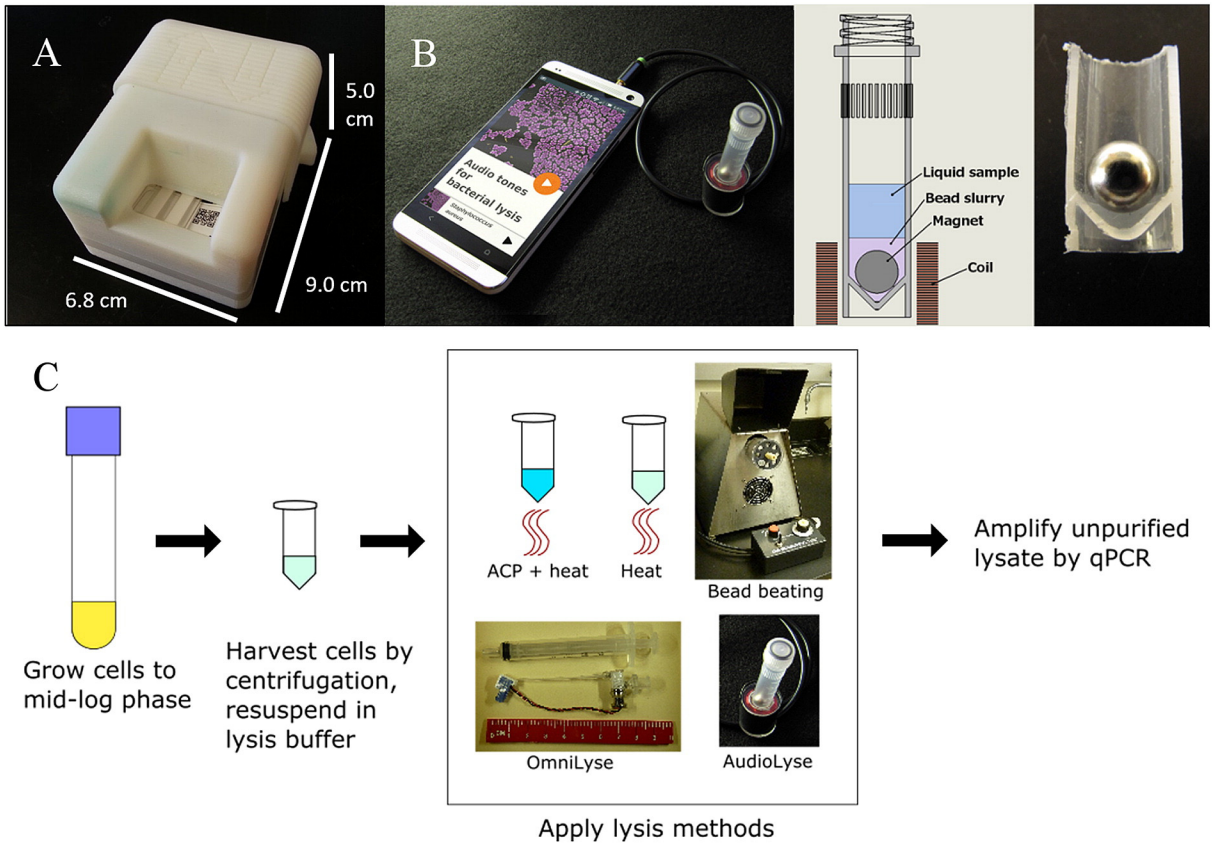

Fig. 1. Point-of-care-compatible lysis of a broad range of pathogens. A) The MAD NAAT platform is a disposable instrument-free pathogen detection device currently being developed for point-of-care medical applications. To solve sample preparation hurdles for this device, we investigated a broad range of pathogen lysis methods. B) The AudioLyse device as used in previous work (Buser et al., 2015b) C) The general lysis procedure followed in this work.

to develop a procedure to release amplifiable DNA or RNA from as broad a range of bacteria and viruses as possible, and allow direct input of lysed sample into the amplification reaction *without a purification step*. To meet, to the extent possible, the "user-friendly" ASSURED principle, we have also tried to minimize the number of user steps necessary for MAD NAAT, including sample preparation.

Lysis methods that use extreme pH, chaotropic salts, organic solvents, or detergents can interfere with subsequent nucleic acid amplification if these chemicals cannot be removed (Atshan et al., 2012; Chomczynski and Rymaszewski, 2006; Eckert and Kunkel, 1990; Sung et al., 2003; Wilson, 1997). Contaminant mitigation relies on purification using laboratory equipment (e.g. centrifuge), or drastic dilution of the sample, consequently reducing test sensitivity. We extensively considered the use of FTA paper, a product available from Whatman that was developed to capture and preserve nucleic acids from a variety of sample types, and has been used for prototype point-of-care NAATs (Bearinger et al., 2011). However, we excluded FTA papers from this study because they must be washed before use in amplification reactions like iSDA (Rajendram et al., 2006), and thus were difficult to integrate into MAD NAAT. Remaining lysis techniques include **mechanical** lysis, **thermal** lysis, and **enzymatic** lysis.

Mechanical methods generally use grinding or shearing forces to lyse bacteria; they do not rely on chemicals that could inhibit nucleic acid amplification. A gold-standard mechanical method, bead beating, has been shown to release more DNA from hard-to-lyse microbes, including *S. aureus*, over other routine clinical laboratory techniques (de Boer et al., 2010). Point-of-care-compatible mechanical lysis methods evaluated in this study include the OmniLyse – a single-use, battery-powered miniature bead mill that has been shown to disrupt difficult-to-lyse bacteria and produce amplifiable DNA (Vandeventer et al., 2011) – and our own laboratory's AudioLyse – a miniature bead mill that uses a portable audio device and electromagnetic coil to rotate a spherical magnet in a bead slurry, grinding the sample and causing lysis (shown in Fig. 1B) (Buser et al., 2015b).

Thermal lysis methods have been used to lyse bacteria, but often require the use of detergents such as Triton X-100 or SDS, or organic solvents such as phenol and chloroform (Atshan et al., 2012; Sung et al., 2003) which may inhibit nucleic acid amplification if not removed. Due to the constraints of our device, we chose to test boiling in a hypotonic solution free of these detergents and solvents as our thermal lysis condition.

Enzymatic lysis routinely utilizes proteinases (e.g., proteinase K) and/or lysozyme, but many important pathogens are resistant to these techniques. For example, *S. aureus*, our initial pathogen target in development of the MAD NAAT device, makes an enzyme—a peptidoglycan O-acetyltransferase, OatA—that determines its resistance to lysozyme (Bera et al., 2005). We chose to test the broadly-applicable achromopeptidase (ACP), a cocktail of proteases and peptidoglycan-specific hydrolases (Li et al., 1997; Masaki et al., 1978; Masaki et al., 1981). Like other proteases, ACP is an inhibitor to downstream amplification. However, those components of ACP that affect both iSDA and PCR can be irreversibly deactivated at temperatures above 80 °C (Buser et al., 2016). A boiling water bath enables thermal deactivation of ACP without any other amenities. ACP activity can be stabilized by dehydration in the presence of preservatives (Buser et al., 2016), and thus shipped worldwide in the absence of refrigeration. Prior to this work, it was unknown how tolerant ACP lysis would be to a range of conditions that might be present in an integrated device, such as exposure to varying ambient temperatures and duration of ACP enzyme deactivation by thermal deactivation allowable before DNA degradation began.

The three bacterial human pathogen targets tested represent a broad range of bacterial types. *Staphylococcus aureus* is a Gram-positive,