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SHORT COMMUNICATION

A molecular transport medium for collection, inactivation, transport, and detection of *Mycobacterium tuberculosis*

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SUMMARY

In many parts of the world, the diagnosis of tuberculosis (TB) has rapidly shifted to molecular detection and sequencing formats. The collection and transport of *Mycobacterium tuberculosis* specimens thus remains a challenging problem where TB is common and the infrastructure required for ensuring sample integrity is lacking. PrimeStore® Molecular Transport Medium (MTM) addresses this problem, rapidly inactivating/killing *M. tuberculosis* while preserving genomic DNA even at elevated temperatures for subsequent downstream molecular analysis.

KEY WORDS: *Mycobacterium tuberculosis*; PrimeStore; molecular detection; real-time PCR

MYCOBACTERIUM TUBERCULOSIS is a highly transmissible, bacterial pathogen with significant morbidity and mortality. Tuberculosis (TB) is a global health problem, with an estimated 8.6 million new cases of TB in 2012.[1] Many of these cases occur in impoverished areas of the world where safe handling of infectious agents is difficult. Multidrug-resistant (MDR-) and extensively drug-resistant TB (XDR-TB) strains have been identified in many parts of the world. The continued increase in reported cases of MDR- and XDR-TB strains underscores a growing need for better specimen transport and subsequent sensitive, reproducible detection.

Detection of *M. tuberculosis* continues to move from traditional culture-based detection to more rapid nucleic acid-based formats. Nucleic acid amplification-based tests such as GeneXpert® (Cepheid, Sunnyvale, CA, USA) and GenoType MTBDR*plus*® line-probe assay (LPA; Hain LifeSciences, Nehren, Germany) are now used extensively for the rapid detection of *M. tuberculosis*.[2,3] Furthermore, next-generation sequencing has been employed for rapid characterization of full-length *M. tuberculosis* genes known to confer antibiotic resistance.[4,5] Collection and transport of clinical specimens constitute an ongoing problem in many areas of the world where electricity or cold chain transport is limited and/or unavailable. There is therefore a need for a collection system that: 1) rapidly inactivates *M. tuberculosis* microbes, and 2) preserves genomic DNA while en route before downstream molecular testing.

PrimeStore® Molecular Transport Medium (MTM; Longhorn Vaccines & Diagnostics, San Antonio, TX, USA) is a collection and transport medium developed for research and detection of infectious agents, and was previously shown to kill/inactivate a wide variety of viral, bacterial, and fungal pathogens while preserving and stabilizing released RNA and DNA at ambient temperature for prolonged periods of time.[6] Using a flocked swab swirled in a primary sputum specimen and subsequently placed into PrimeStore MTM®, high quality nucleic acid was obtained for sensitive detection of *M. tuberculosis* using real-time polymerase chain reaction (PCR), the limit of detection for *M. tuberculosis* being comparable to other real-time PCR assays that required considerably higher volumes of primary samples for testing (N Ismail, personal communication, 2012).

In this report, we investigated: 1) the ability of PrimeStore MTM to inactivate low and high level *M. tuberculosis* inocula, and 2) the integrity of the bacterial DNA using real-time PCR following incubation at both ambient and elevated temperatures.

MATERIALS AND METHODS

Liquid culture of *M. tuberculosis* American Type Culture Collection 35801 (American Type Culture Collection, Manassas, VA, USA) was grown in a shaking bath (200 rpm) for 8 days at 37°C in Middlebrook 7H9 broth (Cat. No. 271310; BD,

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Franklin Lakes, NJ, USA) supplemented with glycerol (Cat. No. G5516; Sigma, St. Louis, MO, USA) and oleic acid, albumin, dextrose and catalase (OADC) enrichment (Cat. No. U89; Hardy, Santa Maria, CA, USA). Culture medium was inoculated with actively growing *M. tuberculosis* colonies picked from Middlebrook 7H10 monoplates (Cat. No. W30; Hardy) previously incubated for up to 21 days at 37°C with 5% carbon dioxide. Approximately 2.7×10^7 colony forming units (cfu)/ml culture was diluted with phosphate buffered saline (PBS; Cat. No. D8537; Sigma) containing 0.5% Tween 80 (Cat. No. P4780; Sigma) to achieve approximately 1.0×10^3 cfu/ml (low concentration) and 1×10^6 cfu/ml (high concentration) for subsequent exposure to PrimeStore MTM (Longhorn Vaccines & Diagnostics): 10^6 cfu/ml correlates to a smear-positive sputum specimen of grade 3+ to 4+, whereas 10^3 cfu/ml correlates to a *M. tuberculosis* sputum smear that is marginally positive (i.e., scanty) or negative.

A sterile flocked swab was used to transfer approximately 150 µl cultured *M. tuberculosis* into 1.5 ml PrimeStore MTM. Selected samples suspended in PrimeStore MTM were heated to 80°C for up to 24 h. Aliquots of 50 µl were removed at 1, 10, 20, 30, 60 and 240 min and 24 h for culture in 1 ml Middlebrook 7H9 broth using standard methods. Liquid cultures were subsequently plated onto 7H10 MiddleBrook agar to determine viability using standard methods. Samples shown to have no *M. tuberculosis* growth in culture after incubation for 3 weeks were shipped from Columbus, OH, to San Antonio, TX, for DNA extraction and real-time PCR.

Total genomic DNA was purified from 200 µl aliquots of PrimeStore MTM containing inactivated *M. tuberculosis* culture using the PrimeXtract™ System according to the manufacturer's protocol (Longhorn Vaccines & Diagnostics). Real-time PCR amplification was performed using an ABI 7500 instrument (Life Technologies, Foster City, CA, USA) and the PrimeMix® *M. tuberculosis* mastermix (Longhorn Vaccines & Diagnostics) containing enzyme, reagents, salts, buffers, and primers that target the highly conserved insertion sequence *6110* region of the *M. tuberculosis* genome. Positive control reactions contained DNA extracted from the H37Rv strain. All reactions were performed in triplicate.

As this study did not involve human subjects, informed consent and ethical approval were not required.

RESULTS AND DISCUSSION

M. tuberculosis swab inoculum viability at low (10^3) and high (10^6) cfu/ml after indicated times of exposure in PrimeStore MTM is summarized in the Table. At 10^3 cfu/ml, no *M. tuberculosis* viability was observed following exposure at either ambient or elevated temperature (80°C) at any time point (Table), and no *M. tuberculosis* growth was observed with the high inoculum, i.e., 10^6 cfu/ml following exposure for $\geqslant$30 min (Table).

Real-time PCR was carried out to determine genomic DNA stability and preservation from inactivated *M. tuberculosis*, i.e., non-viable samples (Table). As indicated, observed PCR cycle threshold (C_T) values were similar at all time-points for both low and high cfu/ml *M. tuberculosis* inocula. This was also the case for samples inactivated in PrimeStore MTM and subsequently held at 80°C for up to 24 h before shipment (Figure). These results indicate that low-level *M. tuberculosis* bacterial inoculum was killed within 1 min, whereas the higher level *M. tuberculosis* inoculum required 30 min in PrimeStore MTM for complete killing (Table). Killing of *M. tuberculosis* is thus dependent on both the quantity of bacteria and exposure time in PrimeStore MTM, with higher concentration *M. tuberculosis* inocula requiring a minimum of 30 min of exposure.

Previous studies have shown that PrimeStore MTM is an efficient medium for inactivation of viruses, fungi, and bacteria including gram-positive *Staphylococcus aureus*.[6] High pathogenic H5N1 and H1N1 at viral loads of 10^6 TCID (tissue culture infective dose) 50/ml have also been safely inactivated in PrimeStore MTM.[6] Using a standard swab for sample collection, PrimeStore MTM was shown to effective-

Table Viability of low and high cfu/ml inocula* of *M. tuberculosis* after exposure to PrimeStore® Molecular Transport Medium

	Low concentration *M. tuberculosis* (10^3 cfu/ml)		High concentration *M. tuberculosis* (10^6 cfu/ml)	
Exposure time	22-25°C	80°C	22-25°C	80°C
1 min	Non-viable	NA	Viable	NA
10 min	Non-viable	NA	Viable	NA
20 min	Non-viable	NA	Viable	NA
30 min	Non-viable	Non-viable	Non-viable	Non-viable
60 min	Non-viable	Non-viable	Non-viable	Non-viable
4 h	Non-viable	Non-viable	Non-viable	Non-viable
24 h	Non-viable	Non-viable	Non-viable	Non-viable

* Samples were performed in triplicate.
NA = not applicable.

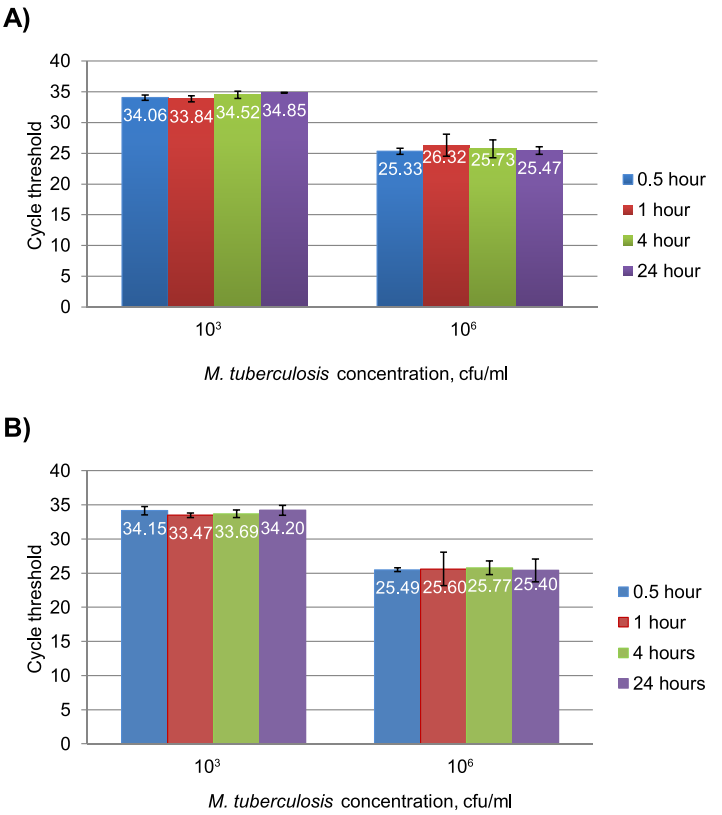

Figure Real-time PCR detection of *M. tuberculosis* in PrimeStore® MTM at indicated exposure time: **A)** ambient temperature (22°–25°C), and **B)** 80°C. *M. tuberculosis* genomic DNA from *M. tuberculosis* was PCR-amplified and real-time cycle threshold values determined as previously described. All determinations were carried out in triplicate with standard error shown. cfu = colony forming unit; PCR = polymerase chain reaction; MTM = molecular transport medium.

ly kill freshly cultured *M. tuberculosis* even at 10^6 cfu/ml, when exposure to PrimeStore MTM was $\geqslant$30 min (Table).

Released *M. tuberculosis* genomic DNA from 10^3 and 10^6 cfu/ml inocula was preserved in PrimeStore MTM at all time points and during subsequent transport. Observed C_T values are consistent with DNA preservation, even at elevated temperatures (80°C for 24 h) before subsequent shipping (Figure). As previously indicated, PrimeStore MTM used with primary sputum samples exhibited efficient killing/inactivation (Nazir et al., 2012, personal communication). These results are consistent with killing/inactivation of low and high level culture *M. tuberculosis* inocula in PrimeStore MTM reported here.

There is currently significant need for safe and efficient transport of specimens, especially in many parts of the world without consistent refrigeration and biosafety level 3 capabilities. PrimeStore MTM thus provides an important tool for collecting and transporting *M. tuberculosis* specimens for subsequent nucleic-acid based detection and gene sequencing, as reproducibility is the foundation of life science research.

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Conflict of interest: none declared.

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RESUME

Dans de nombreuses régions du monde, le diagnostic de tuberculose (TB) est rapidement passé à la détection moléculaire et au séquençage. De ce fait, le recueil et le transport des échantillons de *Mycobacterium tuberculosis* constituent un défi dans les endroits où la prévalence de la TB est élevée et où manquent les infrastructures permettant de garantir l'intégrité des échantillons. Le milieu de transport PrimeStore® Molecular Transport Medium (MTM) répond à ce problème en inactivant/tuant rapidement les isolats de *M. tuberculosis* tout en préservant l'ADN, même à température élevée afin de permettre ensuite l'analyse moléculaire en aval.

RESUMEN

En muchas partes del mundo los medios diagnósticos de la tuberculosis (TB) han evolucionado rápidamente hacia la detección molecular y la secuenciación. Así, la recogida de especímenes de *Mycobacterium tuberculosis* y su transporte continúa planteando problemas en los entornos donde la enfermedad es frecuente y carecen de una infraestructura que procure la integridad de las muestras. El medio de transporte molecular PrimeStore® Molecular Transport Medium ofrece una solución, al inactivar o destruir las cepas de *M. tuberculosis* y al mismo tiempo preservar su ADN genómico, incluso en las altas temperaturas utilizadas luego en el análisis molecular.