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Xpert® MTB/RIF detection of *Mycobacterium tuberculosis* from sputum collected in molecular transport medium

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SUMMARY

BACKGROUND: The Xpert® MTB/RIF assay is widely used for *Mycobacterium tuberculosis* detection. However, specimen transport remains a challenge. PrimeStore Molecular Transport Medium® (PS-MTM) inactivates specimens and stabilizes DNA/RNA at ambient temperature for subsequent molecular detection.

OBJECTIVE: To compare the detection of *M. tuberculosis* concentrations in PS-MTM using Xpert and real-time polymerase chain reaction (RT-PCR), and smear-positive sputum specimens collected using a flocked swab.

METHODS: Dilutions of *M. tuberculosis* in PS-MTM and phosphate buffered saline (PBS) were analyzed using the Xpert assay and commercial RT-PCR. Smear-positive (1+ to 3+) sputum specimens ($n = 17$) were transferred by flocked swab into PS-MTM and PBS, and were compared to standard 1.0 ml sputum Xpert analysis.

RESULTS: Using the Xpert assay, cycle threshold values from high *M. tuberculosis* concentrations in PS-MTM ($>10^3$ colony forming units [cfu]/ml) were increased compared to control. In contrast, *M. tuberculosis* samples containing $<10^3$ cfu/ml, i.e., low concentrations, suspended in PS-MTM resulted in detection down to 10 cfu/ml. Xpert detection efficiency in PS-MTM treated samples (63.2%) was improved compared to PBS controls (34.9%). Xpert detected *M. tuberculosis* in all sputum specimens collected by flocked swabs in PS-MTM, and correlated with routine Xpert detection.

CONCLUSIONS: PS-MTM enhances *M. tuberculosis* detection at low concentrations of *M. tuberculosis*, and provides a simplified and efficient collection method for Xpert detection.

KEY WORDS: PrimeStore MTM; molecular transport medium; tuberculosis testing; Xpert

THE WIDESPREAD USE of the Xpert® MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) has considerably improved the detection of *Mycobacterium tuberculosis* complex (MTC) worldwide. Nowhere is this more evident than in South Africa, which ranks third in global tuberculosis (TB) burden and first in the total number of Xpert assays performed annually.[1,2] In South Africa, Xpert has replaced smear microscopy as the initial diagnostic test in patients with presumed TB.

The Xpert test is a small footprint cartridge-based, real-time polymerase chain reaction (RT-PCR) assay that requires a short (90 min) run-time and can be utilized at point-of-care settings, with a sensitivity superior to that of conventional smear microscopy. However, several TB-related diagnostic challenges remain, including the need for increased sensitivity of detection, and efficient/safe specimen transport from rural locations across Africa and elsewhere in the world.

Previous studies have shown that PrimeStore Molecular Transport Medium® (PS-MTM; Longhorn Vaccines and Diagnostics, San Antonio, TX, USA) is an effective medium for inactivation of viruses, fungi, and bacteria, including *M. tuberculosis*, at concentrations of 10^8 colony forming units [cfu]/ml.[3–5] PS-MTM effectively destroys proteins, lyses lipid membranes, inactivates nucleases, and releases nucleic acids (i.e., RNA/DNA) that are subsequently preserved at ambient temperature for extended periods.[3,4] PS-MTM has been used for shipment and transport of respiratory specimens for influenza detection as well as other pathogens, and its use in RT-PCR and next-generation sequencing (NGS) has been documented.[3–8] *M. tuberculosis* detection was recently performed on clinical sputum specimens collected and transported in PS-MTM at ambient temperature from South Africa to San Antonio, TX, USA, using RT-PCR.[9]

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In the present study, Xpert performance with and without *N*-acetyl-L-cysteine/sodium hydroxide (NALC/NaOH) decontamination was evaluated using serial *M. tuberculosis* dilutions in PS-MTM compared to equivalent *M. tuberculosis* concentrations in phosphate buffered saline (PBS). *M. tuberculosis* was also detected using a panel of *M. tuberculosis*-positive sputum specimens transferred using flocked swab into PS-MTM and PBS, and subsequently compared with routine Xpert analysis using 1.0 ml untreated sputum.

MATERIALS AND METHODS

Preparation of M. tuberculosis *stock*

Glutaraldehyde-killed *M. tuberculosis* was prepared by Battelle (Columbus, OH, USA) as previously described.[3] H37Rv *M. tuberculosis* liquid culture stocks of American Type Culture Collection 35801 (Manassas, VA, USA) were grown in a shaking water bath for 8 days at 37°C in Middlebrook 7H9 broth (BD, Franklin Lakes, NJ, USA) with supplemental nutrients, as previously described.[3]

PrimeStore Molecular Transport Medium®

PS-MTM is a proprietary blend of chemical reagents (denaturants, surfactants, salts, and buffers) optimized for protein destruction, lipid membrane lysis, specimen inactivation, and nucleic acid stabilization.

NALC/NaOH-inactivated M. tuberculosis *dilutions*

For serial 10-fold dilutions, a stock of *M. tuberculosis* culture containing approximately 10^6 cfu/ml *M. tuberculosis* was diluted with PS-MTM or PBS as control (Sigma-Aldrich Corp, St Louis, MO, USA) to achieve a 6-log range from 10^5 to 1 cfu/ml. Negative PBS and positive *M. tuberculosis* controls were also included in the analysis. Prior to testing, *M. tuberculosis* in PS-MTM and PBS was subjected to NALC/NaOH decontamination (2:1, v/v) and tested blindly at the Public Health Laboratory in Milwaukee, WI, USA. For inactivation, NALC/NaOH solution was prepared by the addition of 0.5 g NALC to a 100 ml solution containing 4% (w/v) NaOH and 2.9% (w/v) sodium citrate.

Preparation of M. tuberculosis *dilutions*

At the University of Pretoria (Pretoria, South Africa), *M. tuberculosis* (10^5 cfu/ml) was serially diluted in either PS-MTM or PBS (Sigma-Aldrich Corp) to achieve a broad range of dilutions, i.e., 10^5–10^0 cfu/ml. Negative PBS and positive *M. tuberculosis* controls were also included in the analysis. Triplicate extraction and Xpert testing were performed for each dilution.

Sputum testing

Clinical specimens ($n = 17$) were evaluated from a collection of original sputum specimens previously confirmed as *M. tuberculosis*-positive using smear microscopy and Xpert at the Medical Research Council (University of Pretoria) and the National Institute of Communicable Diseases (Sandringham, South Africa). Smear microscopy was performed as previously described[8] using Ziehl-Neelsen (ZN) stained slides for the detection of acid-fast bacilli (AFB) directly from sputum specimens. Initial *M. tuberculosis* detection was performed within 48 h of specimen collection using Xpert analysis according to the manufacturer's instructions (Instructions of use: Xpert® MTB/RIF GXMTB/RIF-US-10, Cepheid, 2015).

As this retrospective evaluation of *M. tuberculosis* from clinical sputum was performed on decoded specimens, informed consent and ethics approval were not required for the study.

Molecular evaluation of M. tuberculosis *serial dilutions and Xpert*

To evaluate *M. tuberculosis* serial dilutions, DNA extraction and RT-PCR amplification were performed in triplicate as previously described[9] with PrimeXtract™ and PrimeMix® MTB Complex, respectively (Longhorn Vaccines and Diagnostics), using an ABI 7500 instrument (Thermo Fisher, Waltham, MA, USA). Molecular testing in South Africa was carried out using the GeneXpert Dx System with Xpert MTB/RIF Version 4 cartridges (Cepheid), according to the manufacturer's recommendations. The Xpert assay was carried out in triplicate at each dilution, with determination of the average cycle threshold (C_T) constituting quantitative assessment.

For *M. tuberculosis* detection from clinical specimens using the Xpert assay, a flocked swab (PrimeSwab™; Longhorn Vaccines and Diagnostics) was swirled five times in collection cups containing patient sputum, and subsequently transferred to tubes containing 1.5 ml PS-MTM. Although the amount absorbed onto flocked swabs depends on the viscosity and composition of the expectorate, the amount transferred typically ranges from 0.05 to 0.2 ml.[10] Following transfer, the swab stem was broken off and discarded, and the swab, suspended in either PS-MTM or PBS, was vortexed for 10 s, and incubated for 30 min for microbial release and inactivation. Following incubation, a 0.7 ml specimen was placed into 1.4 ml of Xpert sample reagent (2:1 ratio, v/v), vortexed 10–20 times, and incubated for 15 min at room temperature with intermittent shaking. A total of 2 ml (1 ml sputum + 1 ml Xpert diluent) was added to each cartridge and analyzed using the GeneXpert G4 System with Version 4 cartridges according to the manufacturer's instructions. Cartridge sample preparation was processed and analyzed blind, and detection results were subsequently coded and decoded by designated team members. *M. tuberculo-*

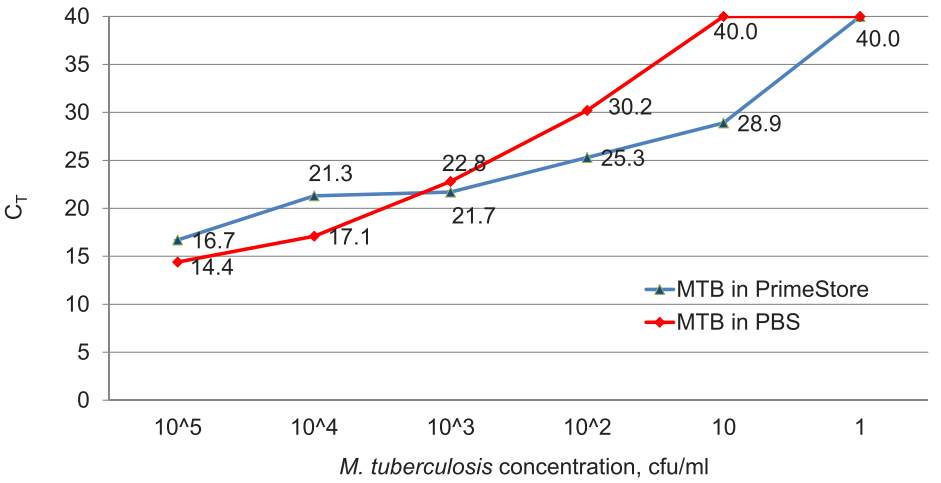

Figure 1 C_T detection values for Xpert® MTB/RIF of the entire *M. tuberculosis* organism treated with *N*-acetyl-L-cysteine/sodium hydroxide from 10-fold dilutions in PrimeStore MTM® (blue triangles), and PBS (red diamonds). C_T 40 = 'no detection'. GeneXpert analysis was performed at the Milwaukee Public Health Department (Milwaukee, WI, USA). C_T = cycle threshold; PBS = phosphate buffered saline. This image can be viewed online in color at http://www.ingentaconnect.com/content/iuatld/ijtld/2016/00000020/00000008/art00023

sis detection was quantitated according to C_T values for GeneXpert probe A, corresponding to high (C_T < 16), medium (C_T 16–22), low (C_T 22–28), and very low (C_T > 28). Resistance to rifampin (RMP), as evidenced by mutation in the *rpo*B gene, is indicated as positive (detected) or negative (not detected). A C_T value of 40 is indicated as 'not detected'.

RESULTS

The Xpert assay detected *M. tuberculosis* from NALC/NaOH pretreated PS-MTM samples in linear fashion over a wide range, detecting down to 10^1 cfu/ml in contrast to 10^2 cfu/ml from the PBS control (Figure 1). Similarly, in non-NALC/NaOH-treated samples, *M. tuberculosis* in PS-MTM was detectable in samples containing 10^1 cfu/ml (3/3 replicates) compared to 10^2 cfu/ml from PBS (Figure 2). At high concentrations, i.e., 10^3 and 10^4 cfu/ml, quantitative C_T detection values for *M. tuberculosis* suspended in PBS were lower, but all were strongly positive (C_T < 23). However, at 10^2 cfu/ml, *M. tuberculosis* suspended in PS-MTM was detected at lower C_T value compared to PBS. Detection of *M. tuberculosis* suspended in PS-MTM was observed at 10^1 cfu/ml ($\chi^2 = 6.0$, $P = 0.14$); however, no detection was observed in PBS (Figures 1 and 2).

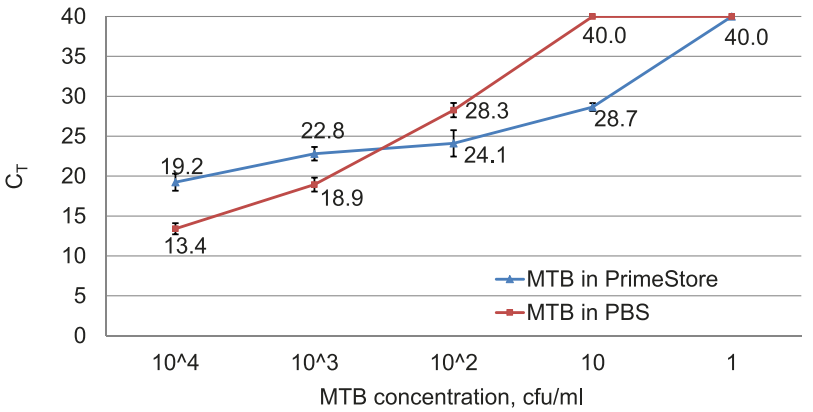

Figure 2 GeneXpert detection of the entire *M. tuberculosis* organism from 10-fold reductions in 1) PrimeStore MTM® (blue triangles), and 2) PBS control (red squares). No *N*-acetyl-L-cysteine/sodium hydroxide decontamination was performed before testing. Triplicate average reactions of quantitative values for GeneXpert Probe A are shown for each dilution, with standard error bar indicated. C_T 40 = 'no detection'. GeneXpert analysis was performed at the National Medical Research Council (Pretoria, South Africa). C_T = cycle threshold; MTB = *Mycobacterium tuberculosis*; PBS = phosphate buffered saline. This image can be viewed online in color at http://www.ingentaconnect.com/content/iuatld/ijtld/2016/00000020/00000008/art00023

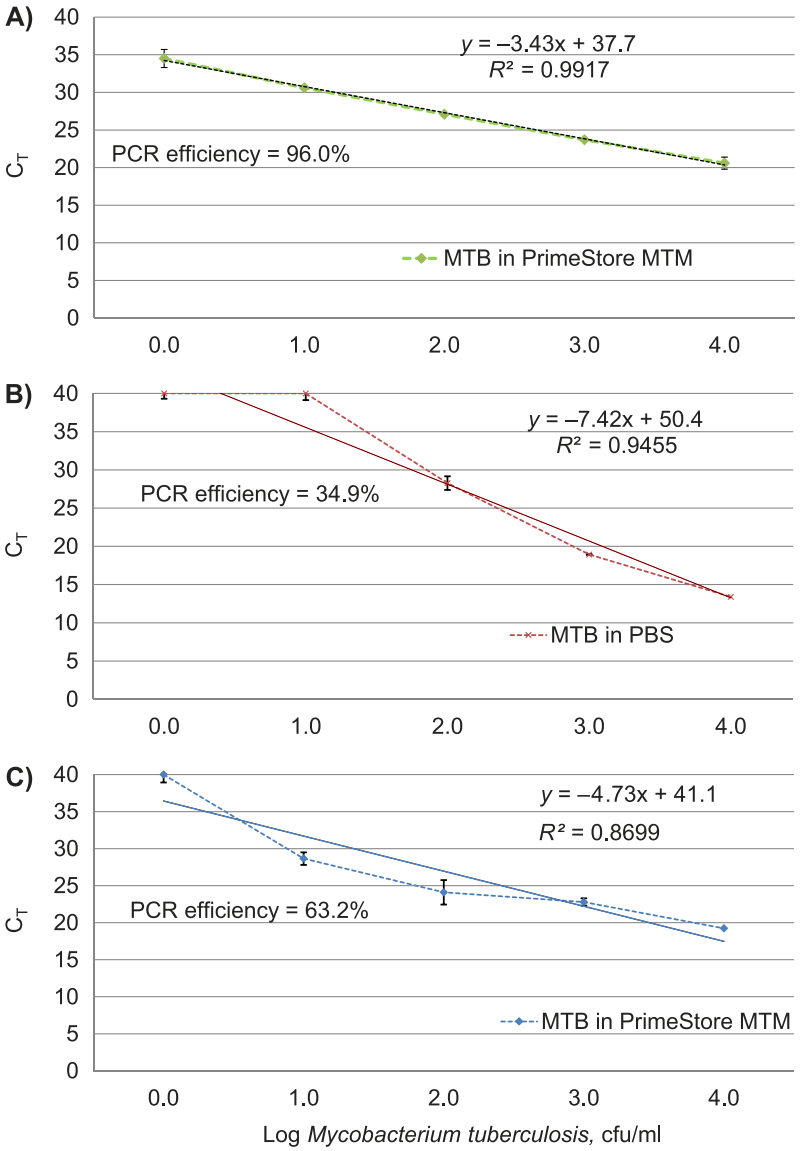

Figure 3 Evaluation of PCR efficiency for *M. tuberculosis* 10-fold serial dilutions performed using **A)** real-time PCR on ABI 7500, **B)** Xpert® MTB/RIF samples of *M. tuberculosis* in PBS, and **C)** Xpert analysis of *M. tuberculosis* in PrimeStore MTM®. Each data point was carried out in triplicate and plotted as the average of those determinations ± the standard error. C_T 40 = 'no detection'. Best-fit linear regression, slope and PCR efficiency are indicated. PCR efficiency (E) = (–1/slope) – 1. C_T = cycle threshold; PCR = polymerase chain reaction; MTB = *Mycobacterium tuberculosis*; PBS = phosphate buffered saline. This image can be viewed online in color at http://www.ingentaconnect.com/content/iuatld/ijtld/2016/00000020/00000008/art00023

To assess *M. tuberculosis* concentrations in PS-MTM (10^0–10^4 cfu/ml), the limit of detection was determined using a commercial RT-PCR assay (Figure 3A). RT-PCR was carried out following standard silica column extraction. *M. tuberculosis* from all concentrations, including the lowest, i.e., 10^0 cfu/ml, was detected. Furthermore, quantitative detection values exhibited linearity and 96% PCR efficiency (Figure 3A). *M. tuberculosis* dilutions suspended in PBS and PS-MTM were evaluated using the GeneXpert Dx MTB/RIF assay system (Figure 3B and 3C). Overall PCR efficiency for *M. tuberculosis* suspended in PBS was 34.9%; however, Xpert *M. tuberculosis* detection efficiency increased significantly, to 63.2%, in PS-MTM suspended samples (Figure 3B and 3C).

The Xpert assay was further assessed using a panel of 17 smear-positive (1+ to 3+) sputum specimens (~0.05–0.2 ml) collected using a flocked swab swirled five times in the sputum, with subsequent placement into either 1.5 ml PS-MTM or 1.5 ml PBS, and ~1.0 ml sputum processed according to the manufacturer's instructions. *M. tuberculosis* from sputum in PS-MTM was detected in 17/17 (100%) specimens compared to routine Xpert testing, i.e., processing ~1.0 ml sputum (Figure 4). Comparison of the Xpert

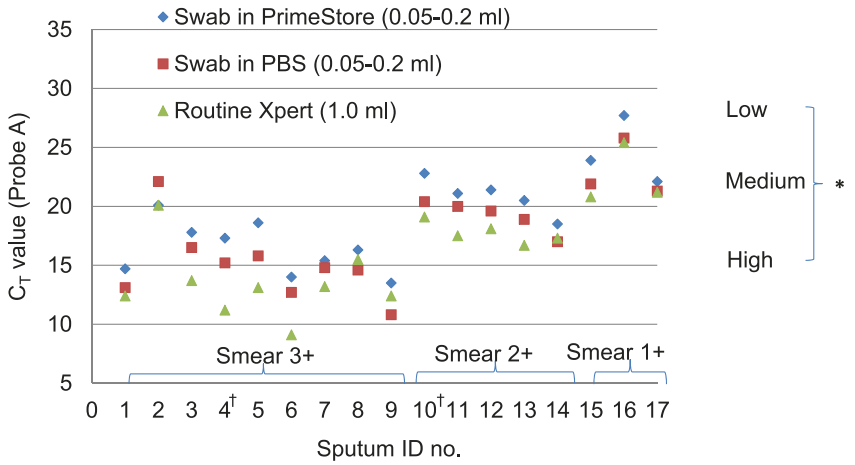

Figure 4 Xpert® MTB/RIF detection of *M. tuberculosis* from smear-positive specimens ($n = 17$). Evaluation of sputum specimens using Xpert was performed using a flocked swab swirled five times in sputum and transfered into 1.5 ml PrimeStore MTM® (blue diamonds) or PBS (red squares). Standard detection was also performed using 1.0 ml sputum (green triangles). For all testing, sample volumes were diluted 2:1 using Xpert dilution buffer according to the manufacturer's recommendation. Smear microscopy bacillary levels for each sample are indicated as 3+, 2+, and 1+.* GeneXpert Dx System quantifies DNA levels according to C_T values: high <16; medium 16–22; low 22–28; and very low >28.† RMP-resistant on Xpert. C_T = cycle threshold; PBS = phosphate buffered saline; RMP = rifampin. This image can be viewed online in color at http://www.ingentaconnect.com/content/iuatld/ijtld/2016/00000020/00000008/art00023

assay using 1.0 ml sputum vs. sputum collected by swab in PS-MTM and PBS indicated similar C_T values, but these were higher for the majority of the PS-MTM specimens (Figure 4). However, variation in C_T values obtained for routine testing and PS-MTM-treated specimens decreased with decreasing smear level, i.e., 3+ to 1+ (Figure 4). In two specimens (4 and 10), the Xpert assay detected RMP resistance in specimens treated with PS-MTM, PBS, as well as routine direct sputum analysis (Figure 4).

DISCUSSION

The Xpert assay has greatly improved *M. tuberculosis* detection, particularly in resource-limited countries.[1] The instrument is simple to operate, and extracts and detects *M. tuberculosis* directly from clinical specimens. However, it is not without limitations. The assay requires a large volume of sputum (minimum 1.0 ml), and the overall efficiency and detection limit of PCR are reduced compared to extraction and subsequent detection using commercial RT-PCR methodology (ABI 7500). However, the collection of small sputum specimen volume(s) suspended in PS-MTM using flocked swab transfer is advantageous for *M. tuberculosis* detection using Xpert. PS-MTM enhances detection 10-fold in samples containing low cfu/ml *M. tuberculosis* (Figures 1 and 2), and improves the overall PCR efficiency of the Xpert assay (Figure 3). At high *M. tuberculosis* concentrations ($\geqslant 10^3$ cfu/ml), PS-MTM did not improve detection, as *M. tuberculosis* nucleic acids from specimens placed in PS-MTM and PBS control were detected. However, in samples containing $<10^2$ cfu/ml, PS-MTM improved Xpert detection of *M. tuberculosis*, consistent with previous studies that have documented rapid membrane lysis and protein (nuclease) destruction to release and preserve the maximum DNA/RNA for detection by PCR.[3–9] Furthermore, the use of small amounts of sputum collected by flocked swab in PS-MTM permits subsequent downstream archiving, retesting, or additional drug resistance profiling by NGS.

NALC/NaOH decontamination is a widely utilized pre-treatment for mycobacterial culture that significantly reduces bacterial competition.[11] Many laboratory practices use NALC/NaOH decontamination before detection for the purpose of safe handling of potentially infectious sputum samples.[11] The Xpert system is applicable to analysis of both raw sputum and NALC/NaOH-decontaminated specimens. In this study, Xpert detection of *M. tuberculosis* samples in PS-MTM was evaluated with and without NALC/NaOH decontamination, with enhanced *M. tuberculosis* detection in 10^2 and 10^1 cfu/ml PS-MTM suspensions compared to PBS (Figures 1 and 2).

Ideally, RT-PCR should be 90–100% efficient ($-3.6 \leqslant$ slope $\leqslant -3.3$). When 100% efficient, C_T values of 10-fold serial dilutions are very close to being 3.3 cycles apart. However, when the slope is <-3.6, PCR efficiency is suboptimal. RT-PCR efficiency is evaluated by generating a 10-fold serial dilution series containing a known number of targets, whereby the slope of the standard curve can be

translated into an efficiency value using the expression:[12]

$$\text{PCR efficiency(E)} = 10^{(-1/\text{slope})} - 1.$$

PCR efficiency for 10-fold *M. tuberculosis* dilutions in PS-MTM using a commercial primer and probe set targeting the multicopy *6110* gene using the ABI 7500 Instrument (Figure 3A) was 96% compared to 63.2% for Xpert (Figure 3C). This difference underscores the overall detection performance between the respective platforms. RT-PCR using the multicopy *6110* target detected *M. tuberculosis* in PS-MTM suspensions down to 1 cfu/ml (Figure 3A). Armand et al. reported that the sensitivity of the Xpert assay was lower than real-time PCR.[13] However, the efficiency and sensitivity of Xpert detection in PS-MTM *M. tuberculosis* suspensions was significantly improved (63.2% and 10 cfu/ml) compared to equivalent PBS suspensions, which exhibited an efficiency of 39.4% and 100 cfu/ml sensitivity (Figure 3B and C).

When Xpert was evaluated using routine 1.0 ml specimens, *M. tuberculosis* was detected in all specimens (17/17), and C_T values were lower than C_T values obtained from swab specimens. However, quantitative detection variation may arise from volume differences. Sputum volume absorbed by swab swirling is approximately 50–200 µl compared to 1.0 ml sputum assessed by routine testing. Variations in C_T values of *M. tuberculosis* suspension between swabs in PS-MTM and routine analysis using 1.0 ml sputum decreased in specimens with lower *M. tuberculosis* levels, as evidenced by reduced smear level (Figure 4).

The use of the Xpert assay with a flocked swab swirled in sputum and subsequent transfer to PS-MTM enables further analysis of drug resistance by NGS with the remaining sample material. In a post-study analysis, selected PS-MTM samples ($n = 5$) were sequenced using MiSeq NGS (Illumina, San Diego, CA, USA). As proof of principle, full genes (*rpo*B, *kat*G, *inh*A, and *pnc*A) were sequenced; 4/5 samples indicated 100% homology to the H37Rv reference strain. One strain exhibited a resistance mutation in the *rpo*B gene (S-531-L) that correlated with resistance detected using Xpert. In addition, a *kat*G mutation in the same strain (S-315-T) was only detected on NGS. While this specimen also exhibited resistance to INH (S-315-T), the detection of RMP resistance alone does not always correlate with INH resistance.[14] Furthermore, cases of INH monoresistance have also been detected.[6,7] Importantly, the opportunity to detect mutations that confer resistance to key antibiotics provides information of considerable importance to improve patient care, track *M. tuberculosis* drug resistance, and facilitate patient selection for new drug development.

The purpose of this study was to analyze Xpert detection using *M. tuberculosis* serial dilutions in PS-MTM and PBS. An evaluation was also performed using a limited panel of smear-positive (1+ to 3+) sputum transferred by flocked swab into PS-MTM, PBS, and by standard 1.0 ml sputum Xpert analysis. Xpert detection was enhanced from serial dilutions and sputum in PS-MTM at *M. tuberculosis* levels of $<10^2$ cfu/ml. An expanded clinical evaluation that includes sputum from scanty and smear-negative specimens is currently underway.

In conclusion, findings reported here indicate that sputum collected in PS-MTM provides a simplified, efficient collection method for Xpert detection, particularly from paucibacillary specimens.

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RESUME

CONTEXTE : Le test Xpert® MTB/RIF est largement utilisé pour la détection de *Mycobacterium tuberculosis*. Cependant, le transport des échantillons reste néanmoins un défi. Le milieu de transport moléculaire PrimeStore® (PS-MTM) inactive les échantillons et stabilise l'ADN/ARN à température ambiante pour la détection moléculaire ultérieure.

BUT : Comparer la détection des concentrations de *M. tuberculosis* dans le milieu PS-MTM avec l'Xpert et la réaction polymérase en chaîne en temps réel (RT-PCR) et des échantillons de crachats à frottis positif recueillis par un écouvillon floqué.

METHODES : Les dilutions de *M. tuberculosis* dans le milieu PS-MTM et la solution saline tamponnée par phosphate (PBS) ont été analysées avec l'Xpert et une RT-PCR commerciale. Les échantillons ($n = 17$) à frottis positif (1+ à 3+) ont été transférés par écouvillon floqué dans le milieu PS-MTM et la PBS et ont été comparés à l'analyse standard de 1,0 ml de crachats par Xpert.

RESULTATS : En recourant au test Xpert, les valeurs CT des concentrations élevées de *M. tuberculosis* dans le milieu PS-MTM ($>10^3$ cfu/ml) ont été augmentées par rapport au témoin. En contraste, les échantillons de *M. tuberculosis* de $<10^3$ cfu/ml, c'est-à-dire des concentrations faibles en suspension dans le milieu PS-MTM, ont permis la détection jusqu'à 10 cfu/ml. L'efficacité de la détection d'Xpert sur des échantillons traités par PS-MTM (63,2%) a été améliorée par comparaison aux témoins PBS (34,9%). L'Xpert a détecté *M. tuberculosis* dans tous les échantillons de crachats recueillis par écouvillons floqués sur milieu PS-MTM et corrélés avec la détection de routine par Xpert.

CONCLUSIONS : Le milieu PS-MTM a amélioré la détection de *M. tuberculosis* à faible concentration et offre une méthode simplifiée et efficace de recueil pour la détection par Xpert.

RESUMEN

MARCO DE REFERENCIA: La prueba Xpert® MTB/RIF se utiliza ampliamente en la detección de *Mycobacterium tuberculosis*. Sin embargo, el transporte de las muestras sigue planteando dificultades. El medio comercial PrimeStore Molecular Transport Medium® (PS-MTM) inactiva las micobacterias y estabiliza el ADN y el ARN a temperatura ambiente para una detección molecular ulterior.

OBJETIVO: Comparar la detección cuantitativa de *M. tuberculosis* en muestras con baciloscopia positiva, recogidas con hisopos de nailon y conservadas en PS-MTM, mediante la prueba Xpert y la reacción en cadena de la polimerasa en tiempo real (RT-PCR).

MÉTODOS: Las diluciones de *M. tuberculosis* en PS-MTM y en solución salina amortiguada por fosfatos (PBS) se analizaron con la prueba Xpert y el estuche comercial de RT-PCR. Las muestras de esputo con baciloscopia positiva (de 1+ a 3+) ($n = 17$) se transfirieron con hisopos de nailon al medio PS-MTM y al PBS y luego se compararon con el análisis corriente de 1,0 ml de esputo con la prueba Xpert.

RESULTADOS: Al utilizar la prueba Xpert, los valores de ciclo umbral en muestras con alta concentración de *M. tuberculosis* ($>10^3$ unidades formadoras de colonias [cfu]/ml) en PS-MTM fueron más altos comparados con el testigo. Al contrario, las muestras que contenían $<10^3$ cfu/ml, es decir bajas concentraciones suspendidas en PS-MTM, permitieron la detección hasta de 10 cfu/ml. La eficacia de la detección de la prueba Xpert en las muestras tratadas con PS-MTM (63,2%) fue mayor que en las muestras de referencia disueltas en PBS (34,9%). La prueba Xpert detectó el *M. tuberculosis* en todas las muestras de esputo recogidas con hisopos de nailon en PS-MTM y exhibió una buena correlación con la detección corriente mediante la prueba Xpert.

CONCLUSIÓN: La utilización del medio de transporte PS-MTM potenció la detección de *M. tuberculosis* en muestras con bajas concentraciones de micobacterias y ofrece un método sencillo y eficiente de recogida de muestras para examen diagnóstico con la prueba Xpert.